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(54) Title: REGULATION OF GENE EXPRESSION BY NEUROLEPTIC AGENTS

(57) Abstract: Polynucleotides, polypeptides, kits and methods are provided related to genes expressed in the central nervous system that are regulated by neuroleptics.

REGULATION OF GENE EXPRESSION BY NEUROLEPTIC AGENTS

(MBHB Case No. 99,022-B)

This application claims priority of U.S. Provisional Application No. 60/161,379, filed October 26, 1999 and U.S. Provisional Application No. 60/186,918, filed on March 3, 2000. Both applications are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

Schizophrenia and dopamine receptors. Midbrain dopamine neurons have been shown to play an important role in normal and diseased brain functions. For example, many psychiatric disorders are associated with overactive dopaminergic activity in the meso-striatal dopamine system which refers to both the nigro-striatal dopamine pathway (neurons linking the substantia nigra to the striatum), and the meso-limbic dopamine pathway (neurons linking the ventral tegmental area to limbic regions, such as amygdala, olfactory tubercle and the nucleus accumbens, which is often considered a ventral extension of the striatum). Additionally, it is known that Parkinson's Disease is caused by the degeneration of dopamine neurons of the nigro-striatal pathway.

Neuroleptic (antipsychotic) drugs. Neuroleptic drugs, such as haloperidol and clozapine, are widely used in the long-term treatment of various psychiatric disorders, including schizophrenia. The antipsychotic effects of neuroleptic drugs are generally attributed to blockade of D_2 receptors in the meso-limbic dopamine system (Metzler et al., *Schizophrenia Bull.*, 2, 19-76 (1976)). The best evidence for this comes from the excellent correlation observed between the therapeutic potency of neuroleptics and their affinity for binding to the D_2 receptor (Seeman et al., *Curr. Opn. Neurol. And Neurosurg.*, 6, 602-608 (1993); Creese et al., *Science*, 192, 481-483 (1976); Peroutka et al., *Am. J. Psych.*, 137, 1518-1522 (1980); Deutch, et al., Schizophren. Res., 4, 121-156 (1991); Seeman, P., Synapse 1, 133-152 (1987)). Although neuroleptic drugs have affinity for other neurotransmitter receptors in the brain, such as muscarinic

acetylcholine, 5-HT, alpha-adrenergic and histamine receptors, no correlation to clinical efficacy has been observed with these receptors (Peroutka et al., *Am. J. Psych.* (1980); Richelson et al., *Eur. J. Pharm.*, 103, 197-204 (1984)).

Studies demonstrate that dopamine receptors become blocked to a level of 70% after only a few hours of neuroleptic treatment (Sedval et al., *Arch. Gen. Psych.*, 43, 995-1006 (1986)). This blockade has been shown to lead to a compensatory increase in dopamine receptor number and supersensitivity of the unblocked receptors (Clow et al., *Psychopharm.*, 69, 227-233 (1980); Rupniak et al., *Life Sci.*, 32, 2289-2311 (1983); Rogue et al., *Eur. J. Pharm.*, 207, 165-169 (1991)). Furthermore, the short-term effects of dopamine antagonists on the brain are well known and include such effects as an increase in dopamine synthesis and catabolism, an increase in the firing rate of dopamine neurons resulting from the inhibition of pre-synaptic dopamine autoreceptors (Grace et al., *J. Pharm. Exp. Ther.*, 238, 1092-1100 (1986), and a potentiation of cyclic AMP formation resulting from the blockade of post-synaptic dopamine receptors (Rupniak et al., *Psychopharm.*, 84, 519-521 (1984)).

Side effects of neuroleptic drugs. In addition to their antipsychotic actions, neuroleptics can cause a series of mild to severe side effects. Some of these side-effects result from the dirty nature of neuroleptic drugs, including hypotension and tachycardia, which results from alpha-adrenergic receptor blockade, and dry mouth and blurred vision, which results from the blockade of muscarinic acetylcholine receptors. The predominant and most undesirable effects that accompany neuroleptic treatment are the long-lasting motor deficits referred to as extrapyramidal side effects (Marsden et al., *Psychol. Med.*, 10, 55-72 (1980)). Extrapyramidal side effects are associated with the blockade of dopamine receptors in the dorsal striatum (Moore et al., *Clin. Neuropharmacol.*, 12, 167-184 (1989) and include such motor deficits as dystonias (muscle spasms), akathisias (motor restlessness), Parkinson's-like symptoms and Tardive Dyskinesia. Roughly 20% of patients taking antipsychotics demonstrate Parkinson's-like symptoms, the blockade of dopamine D_2 receptors in the striatum being functionally equivalent to the degeneration of nigro-striatal dopamine neurons seen in Parkinson's Disease. Tardive Dyskinesia is a

syndrome of abnormal involuntary movements that afflicts roughly 25% of patients on neuroleptic treatment (Jeste et al., *Psychopharmacol.*, 106, 154-160 (1992)). The danger of this side effect is that it can be potentially irreversible, that is, patients can still have symptoms of Tardive Dyskinesia long after the antipsychotic has been discontinued. This implicates an epigenetic component to the effects of chronic neuroleptic treatment.

Interestingly, "typical" neuroleptics, such as haloperidol and fluphenazine, have a much higher propensity for causing extrapyramidal side effects than "atypical" neuroleptic drugs, such as clozapine, which rarely causes these types of effects. Although clozapine differs from haloperidol in its pharmalogical profile, the specific mechanism leading to the lack of motor side effects is unclear. Since clozapine has high affinity for other neurotransmitter receptors, such as muscarinic, adrenergic and serotonin receptors, it is possible that the antipsychotic actions of clozapine are partly due to blockade of these other receptors, which may restore proper balance of the dopamine input and output pathways of the basal ganglia.

Genetics and genes involved in neuropsychiatric disorders. In the general population, the risk for developing a psychiatric disorder is approximately 1-2% (Maier, W., and Schwab, S., Molecular genetics of schizophrenia. *Current Opinion in Psychiatry* 11:19-25 (1998); Kendler, K.S., Twin studies of psychiatric illness: current status and future directions. *Arch Gen Psychiatry* 50:9095-915 (1993)). However, this risk increases to 10% or 40% if one or both parents, respectively, have the disease. Concordance in monozygotic and dizygotic twins remains only as high 40-50% (Maier and Schwab (1998)). While there is undoubtedly a genetic component to the transmission of psychiatric disorders, the lack of full concordance in dizygotic twins indicates that there are other environmental factors that contribute (Maier and Schwab (1998); Kendler (1993)). A current challenge in genetic research on mental illnesses is the identification of mutations conferring susceptibility to, or genes associated with therapeutics for, such disorders. One approach addressing the latter is to identify genes whose expression is altered during the process of drug treatment.

Expression of immediate early genes resulting from acute neuroleptic treatment.

Despite the immediate occupancy of dopamine receptors, neuroleptic drugs have a delayed onset of clinical action, which often can be up to several weeks. Further, as discussed above, neuroleptic drugs are characterized by their ability to cause late and long-lasting motor deficits. The distinct temporal discrepancy which exists between dopamine receptor occupancy and the onset of therapeutic and extrapyramidal side effects, suggests that additional molecular changes in the brain occur downstream from dopamine receptor blockade. In an attempt to identify the downstream molecular mechanisms, studies have focused on dopamine-receptor regulation of individual target genes in the striatum and nucleus accumbens.

For example, several studies have demonstrated that acute treatment with antipsychotic drugs causes induction of several immediate-early genes (Nguyen et al, *Proc. Natl. Acad. Sci.*, 89, 4270-4274 (1992); MacGibbon et al., *Mol. Brain. Res.* 23, 21-32 (1994); Robertson et al., *Neuro. Sci.*, 46, 315-328 (1992); Dragunow et al., *Neuro. Sci.*, 37, 287-294 (1990); Miller *J. Neurochem.*, 54, 1453-1455 (1990)). Some immediate early gene proteins (IEGPs) act as transcription factors by binding to specific DNA sequences and regulating gene transcription. Thus, IEGPs can link receptor-mediated signalling effects to long-term genomic activity. Recent studies have shown that haloperidol, a typical neuroleptic, induces the expression c-Fos in the rat striatum and nucleus accumbens, whereas, clozapine, an atypical neuroleptic, induces c-Fos in the nucleus accumbens only (Nguyen et al., *Proc. Natl. Acad. Sci.* (1992); MacGibbon et al., *Mol. Brain Res.* (1994); Robertson et al., *Neurosci.* (1992)). Haloperidol has also been shown to induce expression of other IEGPs, such as FosB, JunB, JunD and Krox24, in the striatum and nucleus accumbens (Rogue et al., *Brain Res. Bull.* 29, 469-472 (1992); Marsden et al., *Psych. Med.* (1980); Moore et al., *Clin. Neuropharmacol.* (1989)). In contrast, clozapine has been shown to induce Krox24 and JunB in the nucleus accumbens only (Nguyen et al. (1992); MacGibbon et al. (1994)). These results suggest that clozapine's lower tendency to cause extrapyramidal side effects, compared to "typical" neuroleptics, may be associated with its failure to induce IEGPs in the striatum.

The appearance of immediate early genes after acute treatment with neuroleptics likely precedes a number of other molecular changes responsible for the delayed adaptive changes that occur with drug treatment in the striatum.

Changes induced by chronic neuroleptic treatment. Chronic treatment with neuroleptic drugs has also been shown to cause changes in the expression of certain neuropeptides and neurotransmitter receptors. In distinct regions of the striatum, both neurotensin and enkephalin are upregulated after chronic (7 - 28 days) treatment with haloperidol, while levels of protachykinin mRNA are decreased (Merchant et al., *J. Pharm. Exp. Ther.*, 271, 460-471 (1994); Delfs et al., *J. Neurochem.*, 63, 777-780 (1994); Angulo et al., *Neurosci. Lett.* 113, 217-221 (1990)). In contrast, chronic clozapine treatment results in a decrease in enkephalin mRNA levels and only small changes in the expression of neurotensin and tachykinin (Merchant et al. (1994); Mercugliano et al., *Neurosci. Lett.*, 136, 10-15 (1992); Angulo et al. (1990)). These differences suggest that neuropeptides may play a role in the motor deficits that result from treatment with typical neuroleptics.

Researchers have also demonstrated the regulation of genes associated with glutaminergic neurotransmission. For example, a decrease in mRNA expression of the glutamate transporter, GLT-1, was observed in the striatum after 30 days of haloperidol treatment, but not after clozapine exposure (Schneider et al., *Neuroreport.*, 9, 133-136 (1998)). Similar treatment with haloperidol also resulted in an increase in the N-methyl-D-aspartate (NMDA) receptor subunits, NR1 and NR2, whereas clozapine treatment resulted in a lesser induction (Riva et al., *Mol. Brain. Res.* 50, 136-142 (1997)).

In addition, pathological and structural changes in the striatum have been observed after chronic drug treatment. Studies using experimental animals have detected a reduction in the size and number of striatal neurons and neuronal processes, as well as decreases in striatal neuronal density following chronic treatment with haloperidol (Christensen et al., *Acta. Psych. Scand., 46, 14-23 (*1970), Jeste et al., *Psychopharm.*, 106, 154-160 (1992); Mahadik et al., *Biol. Psych.*, 24, 199-217 (1988); Nielson et. al.,

Psychopharm., 59-85-89 (1978). These studies imply that neuroleptics may have a neurotoxic effect on the striatum which could account for the ensuing neuroleptic-induced side effects.

Although the above studies have examined the expression of a few individual target genes, there has been no comprehensive study of the effects of neuroleptics on gene expression over time in the striatum and nucleus accumbens, brain regions considered to be critically involved in the actions of neuroleptic drugs. Thus, the number and identity of the genes which are differentially expressed following acute and chronic treatment with neuroleptics in these tissues remains unknown. Further, there has been no comprehensive examination of the differences between the striatal mRNA expression induced by typical neuroleptics and the expression induced by atypical neuroleptics. Such a comparative study would identify the genes that regulate the antipsychotic actions of neuroleptics versus those responsible for the unwanted side effects associated with these drugs. This information would advance the development of an antipsychotic therapy that would target specific actions of neuroleptic drugs or, alternatively, would selectively block proteins causing the motor side effects.

In addition, a systematic characterization would allow the identification of genes that contribute to neuropathologies associated with neuropsychiatric disorders, such as psychoses, bipolar disorder, and addiction-related behavior. This information can reveal pathways for the mechanism of actions of antipsychotic drugs, as well as provide insight regarding the underlying basis of psychiatric dysfunction. Specifically, the identification of potentially harmful gene products is important to identify molecules that could be useful as diagnostic markers indicating neuropathology. Additionally, the identification of potentially harmful gene products is important to identify molecules that could be amenable to pharmaceutical intervention. A systematic characterization would also allow the identification of beneficial molecules that contribute to conditions of neuroprotection. Such identification of beneficial products could lead to the development of pharmaceutical agents useful in the treatment of neuropsychiatric disorders. Furthermore, the identification of harmful and beneficial products may lead to new lines

of study towards the amelioration of symptoms associated with neuropsychiatric disorders.

Studies have been performed using the PCR-based Total Gene Expression Analysis (TOGA) method to analyze the expression patterns of thousands of genes and compare expression patterns among time courses following clozapine drug treatment. Genes regulated by clozapine treatment were examined in haloperidol-treated animals for a comparative analysis.

SUMMARY OF THE INVENTION

Studies have been performed using the PCR-based Total Gene Expression Analysis (TOGA) method to analyze the expression patterns of thousands of genes and compare expression patterns among time courses following clozapine drug treatment. Genes regulated by clozapine treatment were examined in haloperidol-treated animals for a comparative analysis. TOGA analysis has identified several genes that are altered in their expression in response to clozapine and/or haloperidol administration in mouse brain. In particular, the TOGA system has been used to examine how gene expression in the striatum and nucleus accumbens is regulated by an atypical neuroleptic agent, such as clozapine. These studies have identified proteins and genes which are regulated by the treatment of atypical drugs. Further, these studies have identified at least one gene which is differentially regulated by typical and atypical drugs.

The studies have also examined the pattern of expression of neuroleptic-regulated genes in various regions of the brain. Among other things, these studies are useful to determine the genes specifically associated with anti-psychotic activity versus those associated with extrapyramidal side effects, which information advances the development of improved antipsychotic therapies. The identified neuroleptic-regulated molecules are useful in therapeutic and diagnostic applications in the treatment of various neuropsychiatric disorders, such as psychoses, bipolar disorder, and addiction-related behavior. Such molecules are also useful as probes as described by their size, partial

nucleotide sequence and characteristic regulation pattern associated with neuroleptic administration.

The present invention provides novel polynucleotides and the encoded polypeptides. Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant methods for producing the polynucleotides and the polypeptides. One embodiment of the invention provides an isolated nucleic acid molecule comprising a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107. Also provided is an isolated nucleic acid molecule comprising a polynucleotide at least 95% identical to any one of these isolated nucleic acid molecules and an isolated nucleic acid molecule at least ten bases in length that is hybridizable to any one of these isolated nucleic acid molecules under stringent conditions. Any one of these isolated nucleic acid molecules can comprise sequential nucleotide deletions from either the 5'-terminus or the 3'-terminus. Further provided is a recombinant vector comprising any one of these isolated nucleic acid molecules and a recombinant host cell comprising any one of these isolated nucleic acid molecules. Also provided is the gene corresponding to the cDNA sequence of any one of these isolated nucleic acids.

Another embodiment of the invention provides an isolated polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID

NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107. Also provided is an isolated nucleic acid molecule encoding any of these polypeptides, an isolated nucleic acid molecule encoding a fragment of any of these polypeptides, an isolated nucleic acid molecule encoding a polypeptide epitope of any of these polypeptides, and an isolated nucleic acid encoding a species homologue of any of these polypeptides. Another embodiment of the invention provides an isolated polypeptide of SEQ ID NO: 109. Another embodiment of the invention provides an isolated polypeptide of SEQ ID NO: 110. Preferably, any one of these polypeptides has biological activity. Optionally, any one of the isolated polypeptides comprises sequential amino acid deletions from either the C-terminus or the N-terminus. Further provided is a recombinant host cell that expresses any one of these isolated polypeptides.

Yet another embodiment of the invention comprises an isolated antibody that binds specifically to an isolated polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107. Yet another embodiment of the invention comprises an isolated antibody that binds specifically to an isolated polypeptide of SEQ ID NO: 109. Yet another embodiment of the invention comprises an isolated antibody that binds specifically to an isolated polypeptide of SEQ ID NO: 110. The isolated antibody can be a monoclonal antibody or a polyclonal antibody.

Another embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, such as a neuropsychiatric disorder, comprising administering to a mammalian subject a therapeutically effective amount of a polypeptide of the invention or a polynucleotide of the invention. In one preferred embodiment, a method for preventing, treating, modulating or ameliorating schizophrenia is provided. In another preferred embodiment, a method for preventing, treating, modulating or ameliorating bipolar disorder is provided. In yet another embodiment, a method for preventing, treating, modulating or ameliorating addiction-related behavior is provided.

A further embodiment of the invention provides an isolated antibody that binds specifically to the isolated polypeptide of the invention. A preferred embodiment of the invention provides a method for preventing, treating, modulating, or ameliorating a medical condition, such as a neuropsychiatric disorder, comprising administering to a mammalian subject a therapeutically effective amount of the antibody. In one preferred embodiment, a method for preventing, treating, modulating or ameliorating schizophrenia is provided. In another preferred embodiment, a method for preventing, treating, modulating or ameliorating bipolar disorders is provided. In yet another embodiment, a method for preventing, treating, modulating or ameliorating addiction-related behavior is provided.

An additional embodiment of the invention provides a method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject. The method comprises determining the presence or absence of a mutation in a polynucleotide of the invention. A pathological condition or a susceptibility to a pathological condition, such as a neuropsychiatric disorder, is diagnosed based on the presence or absence of the mutation. In one preferred embodiment, a method for diagnosing schizophrenia is provided. In another preferred embodiment, a method for diagnosing bipolar disorders is provided. In yet another embodiment, a method for preventing, treating, modulating or ameliorating addiction-related behavior is provided.

Even another embodiment of the invention provides a method of diagnosing a pathological condition or a susceptibility to a pathological condition, such as a neuropsychiatric disorder, in a subject. Especially preferred embodiments include methods of diagnosing schizophrenia and bipolar disorders. The method comprises detecting an alteration in expression of a polypeptide encoded by the polynucleotide of the invention, wherein the presence of an alteration in expression of the polypeptide is indicative of the pathological condition or susceptibility to the pathological condition. The alteration in expression can be an increase in the amount of expression or a decrease in the amount of expression. In a preferred embodiment a first biological sample is obtained from a patient suspected of having a neuropsychiatric disorder, for example, schizophrenia, bipolar disorder, or addiction-related behavior, and a second sample from a suitable comparable control source is obtained. The amount of at least one polypeptide encoded by a polynucleotide of the invention is determined in the first and second sample. The amount of the polypeptide in the first and second samples is determined. A patient is diagnosed as having a neuropsychiatric disorder if the amount of the polypeptide in the first sample is greater than or less than the amount of the polypeptide in the second sample.

Another embodiment of the invention provides a method for identifying a binding partner to a polypeptide of the invention. A polypeptide of the invention is contacted with a binding partner and it is determined whether the binding partner effects an activity of the polypeptide.

Yet another embodiment of the invention is a method of identifying an activity of an expressed polypeptide in a biological assay. A polypeptide of the invention is expressed in a cell and isolated. The expressed polypeptide is tested for an activity in a biological assay and the activity of the expressed polypeptide is identified based on the test results.

Still another embodiment of the invention provides a substantially pure isolated DNA molecule suitable for use as a probe for genes regulated in neuropsychiatric

disorders, chosen from the group consisting of the DNA molecules shown in | of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107.

Even another embodiment of the invention provides a kit for detecting the presence of a polypeptide of the invention in a mammalian tissue sample. The kit comprises a first antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to the polynucleotide of the invention or with a polypeptide encoded by the polynucleotide in an amount sufficient for at least one assay and suitable packaging material. The kit can further comprise a second antibody that binds to the first antibody. The second antibody can be labeled with enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, or bioluminescent compounds.

Another embodiment of the invention provides a kit for detecting the presence of genes encoding a protein comprising a polynucleotide of the invention, or fragment thereof having at least 10 contiguous bases, in an amount sufficient for at least one assay, and suitable packaging material.

Yet another embodiment of the invention provides a method for detecting the presence of a nucleic acid encoding a protein in a mammalian tissue sample. A polynucleotide of the invention or fragment thereof having at least 10 contiguous bases is hybridized with the nucleic acid of the sample. The presence of the hybridization product is detected.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present invention will become better understood with reference to the following description, appended claims, and accompanying drawings where:

Figure 1 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases AGTA, showing PCR products produced from mRNA extracted from the striatum/nucleus accumbens of mice treated with 7.5 mg/kg of clozapine for the following durations: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days, where the vertical index line indicates a PCR product of about 106 b.p. that is present in the control sample and enriched in the clozapine-treated samples;

Figure 2A-C is a graphical representation of a more detailed analysis of the 106 bp PCR product indicated in Figure 1. The upper panel (Figure 2A) shows the PCR product generated with the clone specific primer (SEQ ID NO: 28) and the fluoresecnt labeled universal 3' PCR primer (SEQ ID NO: 23). Figure 2B shows the PCR products produced in the original TOGA reaction using a 5' PCR primer, C-G-A-C-G-G-T-A-T-C-G-G-A-G-T-A (SEQ ID NO: 94), and the fluoresecnt labeled universal 3' PCR primer (SEQ ID NO: 23). In the bottom panel (Figure 2C), the traces from the top panel and middle panels are overlaid, demonstrating that the PCR product produced using an extended primer based on the cloned sequence is the same length as the original PCR product;

Figure 3 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases CACC, showing PCR products produced from mRNA extracted from the striatum/nucleus accumbens of mice treated with 7.5 mg/kg of clozapine for the following durations: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days, where the vertical index line indicates a PCR product of

about 201 b.p. that is present in the control sample and increasingly enriched over time in the clozapine-treated samples;

Figure 4 shows a Northern Blot analysis of clone CLZ_5 (CACC 201), where an agarose gel containing poly A enriched mRNA from the striatum/nucleus accumbens of mice treated with clozapine as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_5. Mice were treated with clozapine (7.5 mg/kg) for the following time durations before mRNA extraction: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days;

Figure 5 shows a Northern Blot analysis of clone CLZ_5 (CACC 201), where an agarose gel containing poly A enriched mRNA from the striatum/nucleus accumbens of mice treated with haloperidol as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_5. Mice were treated with haloperidol (4 mg/kg) for the following time durations before mRNA extraction: control (no haloperidol), 45 minutes, 7 hours, 10 days, and 14 days;

Figure 6 is a graphical representation comparing the results of the TOGA analysis of clone CLZ_5 shown in Fig. 3 and the Northern Blot analysis of clone CLZ_5 shown in Figure 4;

Figure 7A-C is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_5, showing the pattern of CLZ_5 mRNA expression in mouse anterior brain (7A), midbrain (7B), and posterior brain (7C), where CLZ_5 is expressed in scattered glial cells and white matter tracts;

Figure 8A-I is an *in situ* hybridization analyses, using an antisense cRNA probe directed against the 3' end of CLZ_5, showing CLZ_5 mRNA expression in mouse anterior brain (8A-C), midbrain (8D-F), and posterior brain (8G-I) in saline-treated mice (top row), mice treated with clozapine for 5 days (middle row), and mice treated with

clozapine for 14 days (bottom row), where the clozapine treatment induces expression in the glial cells;

Figure 9A-H shows a darkfield photomicrograph of various brain regions, including the corpus callosum (cc, Fig. 9A, E); caudate putamen (CPu, Fig. 9B, F); anterior commissure (aca, Fig. 9C, G); and globus pallidus (GP, Fig. 9D, H) in control (9A-D) and clozapine-treated (9E-H) animals;

Figure 10A-D shows a darkfield photomicrograph in the internal capsule (ic) (10A, B) and a brightfield view of the optic tract (opt) (10C, D) from control (10A, C) and clozapine-treated (10B, D) animals;

Figure 11A-H shows GFAP and apoD co-localization in the striatum (11A, B, D, E) and optic tract (11C, F) of control saline (11A, B, C) and clozapine-treated animals (11D, E, F), with thick arrows designating the co-localization of GFAP and apoD mRNA and thin arrows designating the expression of apoD only; 11G-H shows apoD immunohistochemistry with an anti-human apoD primary antibody (Novocastra, Newcastle, UK) in the optic tract of control saline (11G) and clozapine-treated animals (11H).

Figure 12 shows a Northern Blot analysis of clone CLZ_5, where an agarose gel containing poly A enriched mRNA from cultured glial cells treated with clozapine as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_5. Cultured glial cells were treated with different concentrations of clozapine for different lengths of time before mRNA extraction as follows: A= control (no clozapine), B= 100 nM clozapine, 1 day, C= 1μM clozapine, 1 day, D= 100 nM clozapine, 1 week, E= 1μM clozapine, 1 week;

Figure 13 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases TTGT, showing PCR products produced from mRNA extracted from the striatum/nucleus accumbens of mice treated with 7.5 mg/kg clozapine

as follows: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days, where the vertical index line indicates a PCR product of about 266 b.p. that is present in the control sample, is down-regulated within 45 minutes in the clozapine-treated sample, and remains down-regulated for 14 days in the presence of clozapine;

Figure 14 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases TTGT, showing PCR products produced from mRNA extracted from the brain of morphine-treated mice as follows: control striatum (PS), acutely treated striatum (AS), withdrawal striatum (WS), control amygdala (PA), acutely treated amygdala (AA), chronically treated amygdala (TA), and withdrawal amygdala (WA), where the vertical index line indicates a PCR product of about 266 b.p. that is more abundant in control striatum than control amygdala and is differentially regulated by morphine in striatum versus amygdala;

Figure 15 shows a Northern Blot analysis of clone CLZ_40 (TTGT 266), where an agarose gel containing poly A enriched mRNA from the striatum/nucleus accumbens of clozapine-treated mice as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_40. Mice were treated with clozapine (7.5 mg/kg) for the following time durations before mRNA extraction: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days;

Figure 16 is a graphical representation comparing the results of the TOGA analysis of clone CLZ_40 shown in Fig. 13 and the Northern Blot analysis of clone CLZ_40 shown in Figure 15;

Figure 17A-B is an *in situ* hybridization analysis, showing clone CLZ_40 mRNA expression in mouse brain using an antisense cRNA probe directed against the 3' end of CLZ_40, where 17A shows expression in the nucleus accumbens (Acb) and pyriform cortex (Pir) and 17B shows expression in the dentate gyrus (DG);

Figure 18 is a graphical representation of the results of TOGA analysis using a 5' PCR primer with parsing bases TATT, showing PCR products produced from mRNA extracted from the striatum/nucleus accumbens of mice treated with 7.5 mg/kg clozapine as follows: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days, where the vertical index line indicates a PCR product of about 89 b.p. that is present in the control sample and is differentially regulated by clozapine treatment over time.

Figure 19 shows the consensus sequence from the cluster of the following 4 sequences: AI415388: Soares mouse p3NMF19.5 Mus musculus cDNA clone IMAGE:350746 3', mRNA sequence; AI841003: UI-M-AM0-ado-e-04-0-UI.s1 NIH_BMAP_MAM Mus musculus cDNA clone UI-M-AM0-ado-e-04-0-UI 3', mRNA sequence; AI413353: Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:356159 3', mRNA sequence; AI425991: Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:426077 3', mRNA sequence.

Figure 20 shows the sequence of the EST AF006196: Mus musculus metalloprotease-disintegrin MDC15 mRNA, complete cds.

Figure 21 shows the the consensus sequence from the cluster of the following 3 sequences: C86593: Mus musculus fertilized egg cDNA 3'-end sequence, clone J0229E09 3', mRNA sequence; AI428410: Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone IMAGE:553802 3', mRNA sequence; AI561814: Stratagene mouse skin (#937313) Mus musculus cDNA clone IMAGE:1227449 3', mRNA sequence.

Figure 22 is a graphical representation of a Northern Blot analysis of clone CLZ_44 (ACGG 352), where an agarose gel containing poly A enriched mRNA from the striatum/nucleus accumbens of clozapine-treated mice as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_44. Mice were treated with clozapine (7.5 mg/kg), haloperidol (4 mg/kg), or ketanserin (4 mg/kg) for two weeks before mRNA extraction.

Figure 23 is a graphical representation of a Northern Blot analysis of clone CLZ_38 (TGCA 109), where an agarose gel containing poly A enriched mRNA from the striatum/nucleus accumbens of clozapine-treated mice as well as size standards was blotted after electrophoresis and probed with radiolabelled CLZ_38. Mice were treated with clozapine (7.5 mg/kg) for the following time durations before mRNA extraction: control (no clozapine), 45 minutes, 7 hours, 5 days, 12 days, and 14 days;

Figure 24A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_16, showing the pattern of CLZ_16 mRNA expression in coronal sections through hemispheres in mouse brain. Figure 24A shows dense labelling in the cortex and surrounding the hippocampal formation as well as moderate labelling in the dorsal thalamus and posterior brain. Figure 24B shows uniform labelling throughout;

Figure 25A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_17, showing the pattern of CLZ_17 mRNA expression in a coronal section through the hemispheres (25A) and cross section through the midbrain (25B) in mouse brain;

Figure 26A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_24, showing the pattern of CLZ_24 mRNA expression in a coronal section through the hemispheres (26A) and cross section through the brainstem (26B) in mouse brain;

Figure 27A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_26, showing the pattern of CLZ_26 mRNA expression in a coronal section of the hemispheres at the level of hippocampal formation (27A) and coronal section of the hemispheres at the level of striatum (27B) in mouse brain;

Figure 28A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_28, showing the pattern of CLZ_28 mRNA expression in a coronal section through the hemispheres at the level of hippocampus (28A) and coronal section through the posterior region of hemispheres (28B) in mouse brain;

Figure 29A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_3, showing the pattern of CLZ_3 mRNA expression in a coronal section through the hemispheres at level of hippocampus (29A) and cross section through midbrain (29B) in mouse brain;

Figure 30A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_34, showing the pattern of CLZ_34 mRNA expression in a coronal section through the hemispheres at the level of hippocampus (30A) and cross section through the midbrain (30B) in mouse brain;

Figure 31A-C is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_43, showing the pattern of CLZ_43 mRNA expression in coronal sections of the hemispheres showing labelling in the striatum (31A), labelling in the cortex (31B), and intense labelling in the striatum (31C) in mouse brain;

Figure 32A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_44, showing the pattern of CLZ_44 mRNA expression in a coronal section showing labelling in the hippocampus, hypothalamus, and temporal cortex (32A) and coronal section showing cortical labelling (32B) in mouse brain;

Figure 33A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_64, showing the pattern of CLZ_64 mRNA expression in different coronal sections of the hemispheres in mouse brain.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide.

In the present invention, a "secreted" protein refers to those proteins capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as those proteins released into the extracellular space without necessarily containing a signal sequence. If the secreted protein is released into the extracellular space, the secreted protein can undergo extracellular processing to produce a "mature" protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. For example, the polynucleotide can contain all or part of the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107, or the complement thereof, or the cDNA. "Stringent hybridization conditions" refers to an overnight incubation at 42° C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM sodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37°C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50°C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a

complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

A polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without

branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formulation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, e.g., T. E. Creighton, *Proteins – Structure And Molecular Properties*, 2nd Ed., W. H. Freeman and Company, New York (1993); B. C. Johnson, Ed., *Posttranslational Covalent Modification Of Proteins*, Academic Press, New York, pgs. 1-12 (1983); Seifter et al., *Meth. Enzymol*, 182:626-646 (1990); Rattan et al., *Ann. N.Y. Acad. Sci.* 663:48-62 (1992)).

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The translated amino acid sequence, beginning with the methionine, is identified although other reading frames can also be easily translated using known molecular

biology techniques. The polypeptides produced by the translation of these alternative open reading frames are specifically contemplated by the present invention.

SEQ ID NOs: 1-19; 49-52; 57-72 and 107 and the translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from the translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 may be used to generate antibodies which bind specifically to the secreted proteins encoded by the cDNA clones identified.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

The present invention also relates to the genes corresponding to SEQ ID NOs: 1-19; 49-52; 57-72 and 107, and translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologues. Species homologues may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988). Polypeptides of the invention also can be purified from natural or recombinant sources using antibodies of the invention raised against the secreted protein in methods which are well known in the art.

Signal Sequences

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein (*Virus Res.*, 3:271-286 (1985)). The method of von Heinje uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein (*Nucleic Acids Res.*, 14:4683-4690 (1986)). Therefore, from a deduced amino acid sequence, a signal sequence and mature sequence can be identified.

In the present case, the deduced amino acid sequence of the secreted polypeptide was analyzed by a computer program called Signal P (Nielsen et al., *Protein Engineering*, 10:1-6 (1997), which predicts the cellular location of a protein based on the amino acid

sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the present invention provides secreted polypeptides having a sequence corresponding to the translations of SEQ. ID NOs: 1-19 which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

"Identity" per se has an art-recognized meaning and can be calculated using published techniques. (See, e.g., Lesk, A.M., Ed., *Computational Molecular Biology*, Oxford University Press, New York, (1988); Smith, D.W., Ed, *Biocomputing: Informatics And Genome Projects*, Academic Press, New York, (1993); Griffin, A.M., and Griffin, H.G., Eds., *Computer Analysis Of Sequence Data, Part I*, Humana Press, New Jersey, (1994); von Heinje, G., *Sequence Analysis In Molecular Biology*, Academic

Press, (1987); and Gribskov, M. and Devereux, J., Eds., *Sequence Analysis Primer*, M Stockton Press, New York, (1991)). While there exists a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans. (See, e.g., Carillo, H., and Lipton, D., *SIAM J. Applied Math.*, 48:1073 (1988)). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Martin J. Bishop, ed., "Guide to Huge Computers," Academic Press, San Diego, (1994), and Carillo, H., and Lipton, D., SIAM J. Applied Math, 48:1073 (1988)). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux, J., et al., *Nuc. Acids Res.* 12(l):387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., *J. Molec. Biol.*, 215:403 (1990), Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711 (using the local homology algorithm of Smith and Waterman, *Advances in Applied Mathematics* 2:482-489 (1981)).

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, 95% identical to a reference sequence, the parameters are set so that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (*Comp. App. Biosci.*, 6:237-245 (1990)) The term "sequence" includes nucleotide and amino acid sequences. In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB search of a DNA sequence to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=l, Joining Penalty=30, Randomization Group Length=0, and Cutoff Score=l, Gap Penalty=5, Gap Size Penalty 0.05, and Window Size=500 or query sequence length in nucleotide bases, whichever is shorter.

Preferred parameters employed to calculate percent identity and similarity of an amino acid alignment are: Matrix=PAM 150, k-tuple=2, Mismatch Penalty= 1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=l, Gap Penalty=5, Gap Size Penalty=0.05, and Window Size=500 or query sequence length in amino acid residues, whichever is shorter.

As an illustration, a polynucleotide having a nucleotide sequence of at least 95% "identity" to a sequence contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107 means that the polynucleotide is identical to a sequence contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107 or the cDNA except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the total length (not just within a given 100 nucleotide stretch). In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to SEQ ID NOs: 1-19; 49-52; 57-72 and 107, up to 5% of the nucleotides in the sequence contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107 or the cDNA can be deleted, inserted, or substituted with other nucleotides. These changes may occur anywhere throughout the polynucleotide.

Further embodiments of the present invention include polynucleotides having at least 80% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to a sequence contained in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the polynucleotides having at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity will encode a polypeptide identical to an amino acid sequence contained in the translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference polypeptide, is intended that the amino acid sequence of the polypeptide is identical to the reference polypeptide except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the total length of the reference polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be

deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Further embodiments of the present invention include polypeptides having at least 80% identity, more preferably at least 85% identity, more preferably at least 90% identity, and most preferably at least 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence contained in translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Preferably, the above polypeptides should exhibit at least one biological activity of the protein.

In a preferred embodiment, polypeptides of the present invention include polypeptides having at least 90% similarity, more preferably at least 95% similarity, and still more preferably at least 96%, 97%, 98%, or 99% similarity to an amino acid sequence contained in translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons. For instance, a polynucleotide variant may be produced to optimize codon expression for a particular host, i.e., codons in the human mRNA may be changed to those preferred by a bacterial host such as *E. coli*).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism (Lewin, B., Ed., *Genes II*, John Wiley & Sons, New York (1985)). These

allelic variants can vary at either the polynucleotide and/or polypeptide level. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. Ron et al., reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues (*J. Biol. Chem.*, 268: 2984-2988 (1993)). Similarly, interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli et al., *J. Biotechnology*, 7:199-216 (1988)).

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle et al., conducted extensive mutational analysis of human cytokine IL-l a (*J. Biol. Chem.*, 268:22105-22111 (1993)). They used random mutagenesis to generate over 3,500 individual IL- 1 a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators concluded that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See Gayle et al., (1993), Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking

N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, et al., *Science,* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (the introduction of single alanine mutations at every residue in the molecule) can be used (Cunningham and Wells, *Science,* 244:1081-1085 (1989)). The resulting mutant molecules can then be tested for biological activity.

According to Bowie et al., these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile;

replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp; and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as decreased aggregation. As known, aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity (see, e.g., Pinckard et al., *Clin. Exp. Immunol.*, 2:331-340 (1967); Robbins et al., *Diabetes,* 36: 838-845 (1987); Cleland et al., *Crit. Rev. Therapeutic Drug Carrier Systems,* 10:307-377 (1993)).

Polynucleotide and Polypeptide Fragments

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in that shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in that shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. These

nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, and more nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments having a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, to the end of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in the translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Protein fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, or 61 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50 or 60, amino acids in length. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of the translations of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Epitopes & Antibodies

In the present invention, "epitopes" refer to polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a polypeptide fragment comprising an epitope, as well as the polynucleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response (see, e.g., Geysen et al., *Proc. Natl. Acad. Sci. USA,* 81:3998-4002 (1983)).

Fragments which function as epitopes may be produced by any conventional means (see, e.g., Houghten, R. A., *Proc. Natl. Acad. Sci. USA,* 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson et al., *Cell,* 37:767-778 (1984); Sutcliffe, J. G. et al., *Science,* 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al., *Proc. Natl. Acad. Sci.*, USA 82:910-914; and Bittle, F. J. et al., *J. Gen. Virol.*, 66:2347-2354 (1985)). A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.)

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.*, 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Additional embodiments include chimeric antibodies, e.g., humanized versions of murine monoclonal antibodies. Such humanized antibodies may be prepared by known techniques, and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment may comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature,* 332:323, 1988), Liu et al. (*PNAS,* 84:3439, 1987), Larrick et al. (*Bio/Technology,* 7:934, 1989), and Winter and Harris (*TIPS,* 14:139, May, 1993).

One method for producing a human antibody comprises immunizing a non-human animal, such as a transgenic mouse, with a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107, whereby antibodies directed against the polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107 are generated in said animal. Procedures have been developed for generating human antibodies in non-human animals. The antibodies may be partially human, or preferably completely human. Non-human animals (such as transgenic mice) into which genetic material encoding one or more human immunoglobulin chains has been introduced may be employed. Such transgenic mice may be genetically altered in a variety of ways. The genetic manipulation may result in human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains in at least some (preferably virtually all) antibodies produced by the animal upon immunization. Antibodies produced by immunizing transgenic animals with a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107 are provided herein.

Mice in which one or more endogenous immunoglobulin genes are inactivated by various means have been prepared. Human immunoglobulin genes have been introduced into the mice to replace the inactivated mouse genes. Antibodies produced in the animals incorporate human immunoglobulin polypeptide chains encoded by the human genetic material introduced into the animal. Examples of techniques for production and use of such transgenic animals are described in U.S. Patent Nos. 5,814,318; 5,569,825; and 5,545,806, which are incorporated by reference herein.

Monoclonal antibodies may be produced by conventional procedures, e.g., by immortalizing spleen cells harvested from the transgenic animal after completion of the immunization schedule. The spleen cells may be fused with myeloma cells to produce hybridomas by conventional procedures.

A method for producing a hybridoma cell line comprises immunizing such a transgenic animal with an immunogen comprising at least seven contiguous amino acid residues of a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107; harvesting spleen cells from the immunized animal; fusing the harvested spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Such hybridoma cell lines, and mosclonal antibodies produced therefrom, are encompassed by the present invention. Monoclonal antibodies secreted by the hybridoma cell line are purified by conventional techniques.

Antibodies may be employed in an *in vitro* procedure, or administered *in vivo* to inhibit biological activity induced by a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Disorders caused or exacerbated (directly or indirectly) by the interaction of such polypeptides of the present invention with cell surface receptors thus may be treated. A therapeutic method involves *in vivo* administration of a blocking antibody to a mammal in an amount effective for reducing a biological activity induced by a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107. For example, chronic administration of neuroleptics can cause unwanted side effects. Administration of an antibody derived from the identified polynucleotides might block the signaling that causes these side effects. Alternatively, an antibody derived from the identified polynucleotides might selectively block proteins causing motor side effects.

Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to an antibody directed against a polypeptide translated from a nucleotide sequence chosen from SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Examples of such agents are well known, and include but are not limited to diagnostic radionuclides, therapeutic radionuclides, and cytotoxic drugs. The conjugates find use in *in vitro* or *in vivo* procedures.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

In addition, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life *in vivo*. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins (see, EP A 394,827; Traunecker et al., *Nature,* 331:84-86 (1988)). Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric

secreted protein or protein fragment alone (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)).

Similarly, EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (see, e.g., EP-A 0 232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5 (see, D. Bennett et al., *J. Molecular Recognition,* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.,* 270:9459-9471 (1995)).

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al, for instance, hexa-histidine provides for convenient purification of the fusion protein (*Proc. Natl. Acad. Sci. USA,* 86:821-824 (1989)). Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell,* 37:767 (1984)). Other fusion proteins may use the ability of the polypeptides of the present invention to target the delivery of a biologically active peptide. This might include focused delivery of a toxin to tumor cells, or a growth factor to stem cells.

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged *in vitro* using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, 293, Bowes melanoma cells and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, PNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., *Basic Methods In Molecular Biology,* (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

Currently no specific diagnostic markers exist that can be used to prevent or delay psychotic episodes of schizophrenia. The polynucleotides of the present invention can be used as chromosome markers for diagnosis for schizophrenia. A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial

modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NOs: 1-19; 49-52; 57-72 and 107 will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-

sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease . Disease mapping data are found, for example in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library) Assuming one megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. The polynucleotides of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 can be used for this analysis of individual humans.

First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in

some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Both methods rely on binding of the polynucleotide to DNA or RNA. For these techniques, preferred polynucleotides are usually 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (see, Lee et al., *Nucl. Acids Res.*, 6:3073 (1979); Cooney et al., *Science*, 241:456 (1988); and Dervan et al., *Science*, 251:1360 (1991) for discussion of triple helix formation) or to the mRNA itself (see, Okano, *J. Neurochem.*, 56:560 (1991) and *Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988) for a discussion of antisense technique.) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals (Erlich, H., *PCR Technology,* Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class H HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention.

Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., et al., *J. Cell. Biol.*, 101:976-985 (1985); Jalkanen, M., et al., *J. Cell. Biol.*, 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99m}Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying secreted protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, Eds., Masson Publishing Inc. (1982)).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. Psychiatric disorders and treatment of psychiatric disorders with neuroleptics, including schizophrenia, are associated with a dysregulation of neurotransmitter and/or neuropeptide levels that can result in the up- or down regulation of polynucleotides and polypeptides. These changes can be diagnosed or monitored by assaying changes in polypeptide levels in tissue or fluids such as CSF, blook, or in fecal samples.

Moreover, polypeptides of the present invention can be used to treat disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B), to inhibit the activity of a polypeptide (e.g., an oncogene), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF

receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor). Polypeptides can also be used as antigens to trigger immune responses.

Local production of neurotransmitters and neuropeptides modulates many aspects of neuronal function. For example, in schizophrenia overactive neurotransmitter activity is thought to be basis for the psychotic behavior. Administration of an antibody to an overproduced polypeptide can be used to modulate neuronal responses in psychiatric disorders such as schizophrenia.

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Biological Activities

The polynucleotides and polypeptides of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides could be used to treat the associated disease.

Nervous System Activity

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of neuroblasts, stem cells or glial cells. A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the central nervous system or peripheral nervous system, by activating or inhibiting the mechanisms of synaptic transmission, synthesis, metabolism and inactivation of neural transmitters, neuromodulators and trophic factors, expression and incorporation of enzymes, structural proteins, membrane channels and receptors in neurons and glial cells, or altering neural membrane compositions.

The etiology of these deficiencies or disorders may be genetic, somatic (such as cancer or some autoimmune disorders), acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular nervous system disease or disorder. The disorder or disease can be any of Alzheimer's disease, Pick's disease, Binswanger's disease, other senile dementia, Parkinson's disease, parkinsonism, obsessive compulsive disorders, epilepsy, encephalopathy, ischemia, alcohol addiction, drug addiction, schizophrenia, amyotrophic lateral sclerosis, multiple sclerosis, depression, and bipolar manic-depressive disorder. Alternatively, the polypeptide or polynucleotide of the present invention can be used to study circadian variation, aging, or long-term potentiation, the latter affecting the hippocampus. Additionally, particularly with reference to mRNA species occurring in particular structures within the central nervous system, the polypeptide or polynucleotide of the present invention can be used to study brain regions that are known to be involved in complex behaviors, such as learning and memory, emotion, drug addiction, glutamate neurotoxicity, feeding behavior, olfaction, viral infection, vision, and movement disorders.

Immune Activity

A polypeptide or polynucleotide of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the

proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotide or polypeptide of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotide or polypeptide of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. A polypeptide or polynucleotide of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Di George's Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polypeptide or polynucleotide of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotide or polypeptide of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotide or polypeptide of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

A polynucleotide or polypeptide of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells or in some ways results in the induction of tolerance, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease. Schizophrenia has several aspects that suggest an autoimmune component to the disease process. Patients with schizophrenia exhibit immunological abnormalities including hypersecretion of cytokines, presence of antinuclear, anticytoplasmic and antiphospholipid antibodies and a decreased ratio of CD4+/CD8+ cells.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by a polypeptide or polynucleotide of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotide or polypeptide of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a

polypeptide or polynucleotide of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polypeptide or polynucleotide of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1).

Hyperproliferative Disorders

A polypeptide or polynucleotide can be used to treat or detect hyperproliferative disorders, including neoplasms. A polypeptide or polynucleotide of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polypeptide or polynucleotide of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus,

thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic region, skin, soft tissue, spleen, thoracic region, and urogenital system.

Similarly, other hyperproliferative disorders can also be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

Infectious Disease

A polypeptide or polynucleotide of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, the polypeptide or polynucleotide of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response. In the case of schizophrenia, where infectious agents may contribute to the pathology, treatment of patients with a polypeptide or polynucleotide of the present invention might act as a vaccine to trigger a more efficient immune response, altering the course of disease.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention. Examples of viruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not

limited to: arthritis, bronchiollitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsielia, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal. These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide of the present invention include, but not

limited to, the following families: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas. These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), Malaria, pregnancy complications, and toxoplasmosis. A polypeptide or polynucleotide of the present invention can be used to treat or detect any of these symptoms or diseases.

Preferably, treatment using a polypeptide or polynucleotide of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues (see, *Science*, 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament

regeneration would quicken recovery time after damage. A polynucleotide or polypeptide of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotide or polypeptide of the present invention.

Chemotaxis

A polynucleotide or polypeptide of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that a polynucleotide or polypeptide of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, a polynucleotide or polypeptide of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (i.e., an agonist), increase, inhibit (i.e., an antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic (see, Coligan et al., *Current Protocols in Immunology,* 1(2), Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., an active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli.* Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a

labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the invention, (b) assaying a biological activity, and (c) determining if a biological activity of the polypeptide has been altered.

Other Activities

A polypeptide or polynucleotide of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide or polynucleotide of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide or polynucleotide of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide or polynucleotide of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, the response to opiates and opioids, tolerance to opiates and opioids, withdrawal from opiates and opioids, reproductive capabilities (preferably by activin or inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide or polynucleotide of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Other Preferred Embodiments

Where a polynucleotide of the invention is down-regulated and exacerbates a pathological condition, such as psychosis or other neuropsychiatric disorders, the expression of the polynucleotide can be increased or the level of the intact polypeptide product can be increased in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, administering a polynucleotide or polypeptide of the invention to the mammalian subject.

A polynucleotide of the invention can be administered to a mammalian subject by a recombinant expression vector comprising the polynucleotide. A mammalian subject can be a human, baboon, chimpanzee, macaque, cow, horse, sheep, pig, horse, dog, cat, rabbit, guinea pig, rat or mouse. Preferably, the recombinant vector comprises a

polynucleotide shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107 or a polynucleotide which is at least 98% identical to a nucleic acid sequence shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107. Also, preferably, the recombinant vector comprises a variant polynucleotide that is at least 80%, 90%, or 95% identical to a polynucleotide comprising SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

The administration of a polynucleotide or recombinant expression vector of the invention to a mammalian subject can be used to express a polynucleotide in said subject for the treatment of, for example, psychosis or other neuropsychiatric disorder. Expression of a polynucleotide in target cells, including but not limited to cells of the striatum and nucleus accumbens, would effect greater production of the encoded polypeptide. In some cases where the encoded polypeptide is a nuclear protein, the regulation of other genes may be secondarily up- or down-regulated.

There are available to one skilled in the art multiple viral and non-viral methods suitable for introduction of a nucleic acid molecule into a target cell, as described above. In addition, a naked polynucleotide can be administered to target cells. Polynucleotides and recombinant expression vectors of the invention can be administered as a pharmaceutical composition. Such a composition comprises an effective amount of a polynucleotide or recombinant expression vector, and a pharmaceutically acceptable formulation agent selected for suitability with the mode of administration. Suitable formulation materials preferably are non-toxic to recipients at the concentrations employed and can modify, maintain, or preserve, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption, or penetration of the composition. *See Remington's Pharmaceutical Sciences* (18th Ed., A.R. Gennaro, ed., Mack Publishing Company 1990).

The pharmaceutically active compounds (i.e., a polynucleotide or a vector) can be processed in accordance with conventional methods of pharmacy to produce medicinal agents for administration to patients, including humans and other mammals. Thus, the pharmaceutical composition comprising a polynucleotide or a recombinant expression

vector may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions).

The dosage regimen for treating a disease with a composition comprising a polynucleotide or expression vector is based on a variety of factors, including the type or severity of the psychosis or other neuropsychiatric disorders, the age, weight, sex, medical condition of the patient, the route of administration, and the particular compound employed. Thus, the dosage regimen may vary widely, but can be determined routinely using standard methods. A typical dosage may range from about 0.1 mg/kg to about 100 mg/kg or more, depending on the factors mentioned above.

The frequency of dosing will depend upon the pharmacokinetic parameters of the polynucleotide or vector in the formulation being used. Typically, a clinician will administer the composition until a dosage is reached that achieves the desired effect. The composition may therefore be administered as a single dose, as two or more doses (which may or may not contain the same amount of the desired molecule) over time, or as a continuous infusion via implantation device or catheter. Further refinement of the appropriate dosage is routinely made by those of ordinary skill in the art and is within the ambit of tasks routinely performed by them. Appropriate dosages may be ascertained through use of appropriate dose-response data.

The cells of a mammalian subject may be transfected *in vivo*, *ex vivo*, or *in vitro*. Administration of a polynucleotide or a recombinant vector containing a polynucleotide to a target cell *in vivo* may be accomplished using any of a variety of techniques well known to those skilled in the art. For example, U.S. Patent No. 5,672,344 describes an *in vivo* viral-mediated gene transfer system involving a recombinant neurotrophic HSV-1 vector. The above-described compositions of polynucleotides and recombinant vectors can be transfected *in vivo* by oral, buccal, parenteral, rectal, or topical administration as well as by inhalation spray. The term "parenteral" as used herein includes, subcutaneous, intravenous, intramuscular, intrasternal, infusion techniques or intraperitoneally.

While the nucleic acids and/or vectors of the invention can be administered as the sole active pharmaceutical agent, they can also be used in combination with one or more vectors of the invention or other agents. When administered as a combination, the therapeutic agents can be formulated as separate compositions that are given at the same time or different times, or the therapeutic agents can be given as a single composition.

Another delivery system for polynucleotides of the invention is a "non-viral" delivery system. Techniques that have been used or proposed for gene therapy include DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, $CaPO_4$ precipitation, gene gun techniques, electroporation, lipofection, and colloidal dispersion (Mulligan, R., (1993) *Science*, 260 (5110):926-32 (1993)). Any of these methods are widely available to one skilled in the art and would be suitable for use in the present invention. Other suitable methods are available to one skilled in the art, and it is to be understood that the present invention may be accomplished using any of the available methods of transfection. Several such methodologies have been utilized by those skilled in the art with varying success (Mulligan, R., (1993) *Science*, 260 (5110):926-32 (1993)).

Where a polynucleotide of the invention is up-regulated and exacerbates a pathological condition in a mammalian subject, such as psychosis or other neuropsychiatric disorders, the expression of the polynucleotide can be blocked or reduced or the level of the intact polypeptide product can be reduced in order to treat, prevent, ameliorate, or modulate the pathological condition. This can be accomplished by, for example, the use of antisense oligonucleotides or ribozymes. Alternatively, drugs or antibodies that bind to and inactivate the polypeptide product can be used.

Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of gene products of the invention in the cell.

Antisense oligonucleotides can be deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. *See* Brown, (1994) *Meth. Mol. Biol.,* 20:1-8; Sonveaux, (1994) *Meth. Mol. Biol.,* 26:1-72; Uhlmann et al., (1990) *Chem. Rev.,* 90:543-583.

Modifications of gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of a gene of the invention. Oligonucleotides derived from the transcription initiation site, *e.g.*, between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (*e.g.*, Gee et al., in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Precise complementarity is not required for successful complex formation between an antisense oligonucleotide and the complementary sequence of a polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent nucleotides, can provide sufficient targeting specificity for mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular polynucleotide sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a polynucleotide of the invention. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, also can be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. *See, e.g.*, Agrawal et al., (1992) *Trends Biotechnol.*, 10:152-158; Uhlmann et al., (1990) *Chem. Rev.*, 90:543-584; Uhlmann et al., (1987) *Tetrahedron. Lett.*, 215:3539-3542.

Ribozymes are RNA molecules with catalytic activity. *See, e.g.*, Cech, (1987) *Science,* 236:1532-1539; Cech, (1990) *Ann. Rev. Biochem.,* 59:543-568; Cech, (1992) *Curr. Opin. Struct. Biol.,* 2:605-609; Couture & Stinchcomb, (1996) *Trends Genet.,* 12:510-515. Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g.*, Haseloff et al., U.S. Patent 5,641,673). The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Examples include engineered hammerhead motif ribozyme molecules that can specifically and efficiently catalyze endonucleolytic cleavage of specific nucleotide sequences.

The coding sequence of a polynucleotide of the invention can be used to generate ribozymes which will specifically bind to mRNA transcribed from the polynucleotide. Methods of designing and constructing ribozymes which can cleave RNA molecules in *trans* in a highly sequence specific manner have been developed and described in the art (*see* Haseloff et al. (1988) *Nature,* 334:585-591). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (*see, e.g.*, Gerlach et al., EP 321,201).

Specific ribozyme cleavage sites within a RNA target can be identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. Suitability of candidate RNA targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. The nucleotide sequences shown in SEQ ID NOs: 1-19; 49-52; 57-72 and 107 and their complements provide sources of suitable hybridization region sequences. Longer complementary sequences can be used to increase the affinity of the

hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related such that upon hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct. Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce a ribozyme-containing DNA construct into cells in which it is desired to decrease polynucleotide expression. Alternatively, if it is desired that the cells stably retain the DNA construct, the construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art. A ribozyme-encoding DNA construct can include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling transcription of ribozymes in the cells.

As taught in Haseloff et al., U.S. Patent 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Production of Diagnostic Tests

Pathological conditions or susceptibility to pathological conditions, such as psychosis or other neuropsychiatric disorders, can be diagnosed using methods of the invention. Testing for expression of a polynucleotide of the invention or for the presence of the polynucleotide product can correlate with the severity of the condition and can also indicate appropriate treatment. For example, the presence or absence of a mutation in a polynucleotide of the invention can be determined and a pathological condition or a susceptibility to a pathological condition is diagnosed based on the presence or absence of the mutation. Further, an alteration in expression of a polypeptide encoded by a polynucleotide of the invention can be detected, where the presence of an alteration in

expression of the polypeptide is indicative of the pathological condition or susceptibility to the pathological condition. The alteration in expression can be an increase in the amount of expression or a decrease in the amount of expression.

As an additional method of diagnosis, a first biological sample from a patient suspected of having a pathological condition, such as psychosis or addiction-related behavior, is obtained along with a second sample from a suitable comparable control source. A biological sample can comprise saliva, blood, cerebrospinal fluid, amniotic fluid, urine, feces, or tissue, such as gastrointestinal tissue. A suitable control source can be obtained from one or more mammalian subjects that do not have the pathological condition. For example, the average concentrations and distribution of a polynucleotide or polypeptide of the invention can be determined from biological samples taken from a representative population of mammalian subjects, wherein the mammalian subjects are the same species as the subject from which the test sample was obtained. The amount of at least one polypeptide encoded by a polynucleotide of the invention is determined in the first and second sample. The amounts of the polypeptide in the first and second samples are compared. A patient is diagnosed as having a pathological condition if the amount of the polypeptide in the first sample falls in the range of samples taken from a representative group of patients with the pathological condition.

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 80%, preferably at least 85%, more preferably at least 90%, most preferably at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the clone sequence and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the start codon and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Similarly preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 in the range of positions beginning with the nucleotide at about the position of the 5' nucleotide of the first amino acid of the signal peptide and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 beginning with the nucleotide at about the position of the 5' nucleotide of the first amino acid of the signal peptide and ending with the nucleotide at about the position of the 3' nucleotide of the clone sequence as defined for SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. In another embodiment, the present invention provides a method for detecting in a biological sample a nucleic acid molecule

comprising a nucleotide sequence which is at least 95% identical to a complete nucleotide sequence chosen from the group consisting of SEQ ID NOs: 1-19; 49-52; 57-72 and 107, which method comprises the steps of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107, which method comprises the steps of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of. a nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NOs: 1-19; 49-52; 57-72 and 107. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in an amino acid sequence translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in acids in an amino acid sequence translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107, in the range of positions beginning with the residue at about the position of the first amino acid of the secreted portion and ending with the residue at about the last amino acid of the open reading frame.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in an amino acid sequence translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in an amino acid sequence translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to acids in an amino acid sequence translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107, which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous

amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encodes a polypeptide comprising an amino acid sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a secreted portion of a human secreted protein comprising an amino acid sequence selected from the group consisting of amino acid sequences translated from SEQ ID NOs: 1-19; 49-52; 57-72 and 107. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a secreted protein activity, which method comprises administering to such an

individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

The present invention also includes a diagnostic system, preferably in kit form, for assaying for the presence of the polypeptide of the present invention in a body sample, such brain tissue, cell suspensions or tissue sections, or body fluid samples such as CSF, blood, plasma or serum, where it is desirable to detect the presence, and preferably the amount, of the polypeptide of this invention in the sample according to the diagnostic methods described herein.

In a related embodiment, a nucleic acid molecule can be used as a probe (an oligonucleotide) to detect the presence of a polynucleotide of the present invention, or a gene corresponding to a polynucleotide of the present invention, or a mRNA in a cell that is diagnostic for the presence or expression of a polypeptide of the present invention in the cell. The nucleic acid molecule probes can be of a variety of lengths from at least about 10, suitably about 10 to about 5000 nucleotides long, although they will typically be about 20 to 500 nucleotides in length. Hybridization methods are extremely well known in the art and will not be described further here.

In a related embodiment, detection of genes corresponding to the polynucleotides of the present invention can be conducted by primer extension reactions such as the polymerase chain reaction (PCR). To that end, PCR primers are utilized in pairs, as is well known, based on the nucleotide sequence of the gene to be detected. Preferably the nucleotide sequence is a portion of the nucleotide sequence of a polynucleotide of the present invention. Particularly preferred PCR primers can be derived from any portion of a DNA sequence encoding a polypeptide of the present invention, but are preferentially from regions which are not conserved in other cellular proteins.

Preferred PCR primer pairs useful for detecting the genes corresponding to the polynucleotides of the present invention and expression of these genes are described in

the Examples, including the corresponding Tables. Nucleotide primers from the corresponding region of the polypeptides of the present invention described herein are readily prepared and used as PCR primers for detection of the presence or expression of the corresponding gene in any of a variety of tissues.

The diagnostic system includes, in an amount sufficient to perform at least one assay, a subject polypeptide of the present invention, a subject antibody or monoclonal antibody, and/or a subject nucleic acid molecule probe of the present invention, as a separately packaged reagent.

In another embodiment, a diagnostic system, preferably in kit form, is contemplated for assaying for the presence of the polypeptide of the present invention or an antibody immunoreactive with the polypeptide of the present invention in a body fluid sample such as for monitoring the fate of therapeutically administered the polypeptide of the present invention or an antibody immunoreactive with the polypeptide of the present invention. The system includes, in an amount sufficient for at least one assay, a polypeptide of the present invention and/or a subject antibody as a separately packaged immunochemical reagent.

Instructions for use of the packaged reagent(s) are also typically included.

As used herein, the term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene or polycarbonate), paper, foil and the like capable of holding within fixed limits a polypeptide, polyclonal antibody or monoclonal antibody of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated polypeptide or antibody or it can be a microtiter plate well to which microgram quantities of a contemplated polypeptide or antibody have been operatively affixed, i.e., linked so as to be capable of being immunologically bound by an antibody or antigen, respectively.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/ sample admixtures, temperature, buffer conditions and the like.

A diagnostic system of the present invention preferably also includes a label or indicating means capable of signaling the formation of an immunocomplex containing a polypeptide or antibody molecule of the present invention.

The word "complex" as used herein refers to the product of a specific binding reaction such as an antibody-antigen or receptor-ligand reaction. Exemplary complexes are immunoreaction products.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyante (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca,

"Immunofluorescence Analysis", in *Antibody As a Tool*, Marchalonis, et al., Eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference. Other suitable labeling agents are known to those skilled in the art.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-amino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I. Another group of useful labeling means are those elements such as ^{11}C, ^{18}F, ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such ^{111} indium or ^{3}H.

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium (see, e.g., Galfre et al., *Meth. Enzymol.*, 73:3-46 (1981)). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable (see, e.g.,Aurameas, et al., *Scand. J. Immunol.*, Vol. 8 Suppl. 7:7-23 (1978); Rodwell et al., *Biotech.*, 3:889-894 (1984); and U.S. Pat. No. 4,493,795).

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of

selectively binding a reagent species of the present invention or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention. Exemplary specific binding agents are second antibody molecules, complement proteins or fragments thereof, *S. aureus* protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of the polypeptide of the present invention in a sample. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Sites et al., *Basic and Clinical Immunology,* 4th Ed., Lange Medical Publications, Los Altos, CA (1982) and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

Thus, in some embodiments, an polypeptide of the present invention, an antibody or a monoclonal antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides can be used that are well known to those skilled in the art. Exemplary adsorption methods are described herein.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron (μm) to about 5 millimeters (mm) in diameter available from several suppliers, e.g., Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

EXAMPLE 1
Identification and Characterization of Polynucleotides Regulated by Neuroleptic Drugs

Male C57Bl/6J mice (20-28 g) were housed in groups of four on a standard 12/12 hour light-dark cycle with ad libitum access to standard laboratory chow and tap water. For the experimental paradigms, mice were divided into groups of 25 and subjected to the following treatments:

Control groups: Mice received a single injection of sterile saline (0.1 ml volume), or no injection, and were sacrificed after 45 minutes.

Acute neuroleptic treatment: Mice received a single intraperitoneal injection of the atypical neuroleptic clozapine (7.5 mg/kg). Animals were sacrificed after 45 minutes.

Chronic neuroleptic treatment: Mice received daily subcutaneous injections of clozapine (7.5 mg/kg) for time periods of 5 days to 2 weeks.

All animals were sacrificed in their cages with CO_2 at the indicated times. Brains were rapidly removed and placed on ice. The striatum, including the nucleus accumbens, were dissected out and placed in ice-cold phosphate-buffered saline.

Isolated RNA was analyzed using a method of simultaneous sequence-specific identification of mRNAs known as TOGA (TOtal Gene expression Analysis) described in Sutcliffe et al. *Proc. Natl. Acad. Sci. USA*, 97(5):1976-1981 (2000); International published application WO 026406; U.S. Patent No. 5,459,037; U.S. Patent No. 5,807,680; U.S. Patent No. 6,030,784; U.S. Patent No. 6,096,503 and U.S. Patent 6,110,680, hereby incorporated herein by reference. Preferably, prior to the application of the TOGA technique, the isolated RNA was enriched to form a starting polyA-containing mRNA population by methods known in the art. In a preferred embodiment, the TOGA method further comprised an additional PCR step performed using four 5' PCR primers in four separate reactions and cDNA templates prepared from a population of antisense cRNAs. A final PCR step that used 256 5' PCR primers in separate reactions produced PCR products that were cDNA fragments that corresponded to the 3'-region of the starting mRNA population. The produced PCR products were then identified by: a) the initial 5' sequence comprising the sequence remainder of the recognition site of the restriction endonuclease used to cut and isolate the 3' region plus the sequence of the preferably four parsing bases immediately 3' to the remainder of the recognition site, preferably the sequence of the entire fragment, and b) the length of the fragment. These two parameters, sequence and fragment length, were used to compare the obtained PCR products to a database of known polynucleotide sequences. Since the length of the obtained PCR products includes known vector sequences at the 5' and 3' ends of the

insert, the sequence of the insert provided in the sequence listing is shorter than the fragment length that forms part of the digital address.

The method yields Digital Sequence Tags (DSTs), that is, polynucleotides that are expressed sequence tags of the 3' end of mRNAs. DSTs that showed changes in relative levels as a result of clozapine treatment were selected for further study. The intensities of the laser-induced fluorescence of the labeled PCR products were compared across samples isolated from the striatum/nucleus accumbens of mice treated with clozapine for 45 minutes, 7 hours, 5 days, 12 days, or 14 days.

In general, double-stranded cDNA is generated from poly(A)-enriched cytoplasmic RNA extracted from the tissue samples of interest using an equimolar mixture or set of all 48 5'-biotinylated anchor primers to initiate reverse transcription. One such suitable set is G-A-A-T-T-C-A-A-C-T-G-G-A-A-G-C-G-G-C-C-G-C-A-G-G-A-A-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-T-V-N-N (SEQ ID NO: 20), where V is A, C or G and N is A, C, G or T. One member of this mixture of 48 anchor primers initiates synthesis at a fixed position at the 3' end of all copies of each mRNA species in the sample, thereby defining a 3' endpoint for each species, resulting in biotinylated double stranded cDNA.

Each biotinylated double stranded cDNA sample was cleaved with the restriction endonuclease MspI, which recognizes the sequence CCGG. The resulting fragments of cDNA corresponding to the 3' region of the starting mRNA were then isolated by capture of the biotinylated cDNA fragments on a streptavidin-coated substrate. Suitable streptavidin-coated substrates include microtitre plates, PCR tubes, polystyrene beads, paramagnetic polymer beads and paramagnetic porous glass particles. A preferred streptavidin-coated substrate is a suspension of paramagnetic polymer beads (Dynal, Inc., Lake Success, NY).

After washing the streptavidin-coated substrate and captured biotinylated cDNA fragments, the cDNA fragment product was released by digestion with NotI, which

cleaves at an 8-nucleotide sequence within the anchor primers but rarely within the mRNA-derived portion of the cDNAs. The 3' MspI-NotI fragments, which are of uniform length for each mRNA species, were directionally ligated into ClaI- NotI-cleaved plasmid pBC SK^+ (Stratagene, La Jolla, CA) in an antisense orientation with respect to the vector's T3 promoter, and the product used to transform *Escherichia coli* SURE cells (Stratagene). The ligation regenerates the NotI site, but not the MspI site, leaving CGG as the first 3 bases of the 5' end of all PCR products obtained. Each library contained in excess of 5×10^5 recombinants to ensure a high likelihood that the 3' ends of all mRNAs with concentrations of 0.001% or greater were multiply represented. Plasmid preps (Qiagen) were made from the cDNA library of each sample under study.

An aliquot of each library was digested with MspI, which effects linearization by cleavage at several sites within the parent vector while leaving the 3' cDNA inserts and their flanking sequences, including the T3 promoter, intact. The product was incubated with T3 RNA polymerase (MEGAscript kit, Ambion) to generate antisense cRNA transcripts of the cloned inserts containing known vector sequences abutting the MspI and NotI sites from the original cDNAs.

At this stage, each of the cRNA preparations was processed in a three-step fashion. In step one, 250ng of cRNA was converted to first-strand cDNA using the 5' RT primer (A-G-G-T-C-G-A-C-G-G-T-A-T-C-G-G, (SEQ ID NO: 21). In step two, 400 pg of cDNA product was used as PCR template in four separate reactions with each of the four 5' PCR primers of the form G-G-T-C-G-A-C-G-G-T-A-T-C-G-G-N (SEQ ID NO: 22), each paired with a "universal" 3' PCR primer G-A-G-C-T-C-C-A-C-C-G-C-G-G-T (SEQ ID NO: 23) to yield four sets of PCR reaction products ("N1 reaction products").

In step three, the product of each subpool was further divided into 64 subsubpools (2ng in 20µl) for the second PCR reaction. This PCR reaction comprised adding 100 ng of the fluoresceinated "universal" 3' PCR primer (SEQ ID NO: 23) conjugated to 6-FAM and 100 ng of the appropriate 5' PCR primer of the form C-G-A-C-G-G-T-A-T-C-G-G-N-N-N-N (SEQ ID NO: 24), and using a program that included an annealing step at a

temperature X slightly above the T_m of each 5' PCR primer to minimize artifactual mispriming and promote high fidelity copying. Each polymerase chain reaction step was performed in the presence of TaqStart antibody (Clonetech).

The products ("N4 reaction products") from the final polymerase chain reaction step for each of the tissue samples were resolved on a series of denaturing DNA sequencing gels using the automated ABI Prizm 377 sequencer. Data were collected using the GeneScan software package (ABI) and normalized for amplitude and migration. Complete execution of this series of reactions generated 64 product subpools for each of the four pools established by the 5' PCR primers of the first PCR reaction, for a total of 256 product subpools for the entire 5' PCR primer set of the second PCR reaction.

The mRNA samples from each timepoint as described above were analyzed. Table 1 is a summary of the expression levels of 495 mRNAs determined from cDNA. These cDNA molecules are identified by their digital address, that is, a partial 5' terminus nucleotide sequence coupled with the length of the molecule, as well as the relative amount of the molecule produced at different time intervals after treatment. The 5' terminus partial nucleotide sequence is determined by the recognition site for MspI (CC GG) and the nucleotide sequence of the parsing bases of the 5' PCR primer used in the final PCR step. The digital address length of the fragment was determined by interpolation on a standard curve and, as such, may vary ± 1-2 b.p. from the actual length as determined by sequencing.

For example, the entry in Table 1 that describes a DNA molecule identified by the digital address MspI AGTA, is further characterized as having a 5' terminus partial nucleotide sequence of CGGAGTA and a digital address length of 106 b.p. The DNA molecule identified as MspI AGTA 106 is further described as being expressed at increasing levels after both acute and chronic treatment with clozapine (see Fig. 1). Additionally, the DNA molecule identified as MspI AGTA 106 is described by its nucleotide sequence, which coresponds with SEQ ID NO: 1.

Similarly, the other DNA molecules identified in Table 1 by their MspI digital addresses are further characterized by: 1) the level of gene expression in the striatum/nucleus accumbens of mice without clozapine treatment (control), 2) the level of gene expression in the striatum/nucleus accumbens of mice treated with clozapine for 45 minutes, 3) the level of gene expression in the striatum/nucleus accumbens of mice treated with clozapine for 7 hours, 4) the level of gene expression in the striatum/nucleus accumbens of mice treated with clozapine for 5 days, 5) the level of gene expression in the striatum/nucleus accumbens of mice treated with clozapine for 12 days, 6) the level of gene expression in the striatum/nucleus accumbens of mice treated with clozapine for 14 days.

Some products, which were also differentially represented, appeared to migrate in positions that suggest that the products were novel based on comparison to data extracted from GenBank. The sequences of such products were determined by one of two methods: cloning or direct sequencing of the PCR products.

Additionally, several of the isolated clones were further characterized as shown in Table 2 and their nucleotide sequences are provided as SEQ ID NOs: 1-19; 49-52; 57-72 and 107 in the Sequence Listing below.

The sequences of SEQ ID NOs: 1-19; 49-52; 57-72 and 107 have had the MspI site found in the native state of the corresponding RNA indicated by the addition of a "C" to the 5' of the sequence. As noted above, the ligation of the sequence into a vector does not regenerate the MspI site; the experimentally determined sequence reported herein has C-G-G as the first bases of the 5' end.

The data shown in Figure 1 were generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-G-T-A; SEQ ID NO: 94) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using

GeneScan software (Perkin-Elmer). The sequences of the PCR products were determined using standard techniques.

The results of TOGA analysis using a 5' PCR primer with parsing bases AGTA (SEQ ID NO.: 94) are shown in Figure 1, which shows the PCR products produced from mRNA isolated from the striatum/nucleus accumbens of mice treated with clozapine for various lengths of time as described above. The vertical index line indicates a PCR product of about 106 b.p. that is present in control cells, and whose expression increases when the striatum/nucleus accumbens of mice are treated with clozapine for 45 minutes, 7 hours, 5 days, 12 days, and 14 days.

Cloning DSTs Without A Candidate Match and Verification of the Cloned DSTs Using Extended TOGA Method

In suitable cases, the PCR product was isolated, cloned into a TOPO vector (Invitrogen) and sequenced on both strands. The database matches for each cloned DST sequence are listed in Table 2. In order to verify that the cloned product corresponds to the TOGA peak of interest, the extended TOGA assay was performed for each DST. PCR primers were designed based on the determined sequences and PCR was performed using the N1 PCR reaction products as a substrate. Oligonucleotides were synthesized with the sequence G-A-T-C-G-A-A-T-C extended at the 3' end with a partial MspI site (C-G-G), and an additional 18 adjacent nucleotides from the determined sequence of the cloned PCR product or DST. For example, for the PCR product with the digital address MspI AGTA 106 (SEQ ID NO: 1), the 5' PCR primer was G-A-T-C-G-A-A-T-C-C-G-G-A-G-T-A-C-A-G-T-G-A-C-T-T-T-G-A-G-T (SEQ ID NO: 28). This 5' PCR primer was paired with the fluorescent labeled universal 3' PCR primer (SEQ ID NO: 23) in a PCR reaction using the PCR N1 reaction product as substrate.

The length of the PCR product generated with the clone specific primer (SEQ ID NO: 28) was compared to the length of the original PCR product that was produced in the TOGA reaction as shown in Figure 2. For CLZ_3 (SEQ ID NO: 1), the upper panel

(Figure 2A) shows the PCR product generated with the clone specific primer (SEQ ID NO: 28) and the fluorescent labeled universal 3' PCR primer (SEQ ID NO: 23). Figure 2B shows the PCR products produced in the original TOGA reaction using a 5' PCR primer C-G-A-C-G-G-T-A-T-C-G-G-A-G-T-A (SEQ ID NO: 94), and the fluorescent labeled universal 3' PCR primer. In the bottom panel (Figure 2C), the traces from the top and middle panels are overlaid, demonstrating that the PCR product produced using an extended primer based on the cloned sequence is the same length as the original PCR product. Other DST clones verified using this method include cases (CLZ_5, SEQ ID NO: 2; CLZ_8, SEQ ID NO: 3; CLZ_10, SEQ ID NO: 4; CLZ_12, SEQ ID NO: 5; CLZ_15, SEQ ID NO: 6; CLZ_24, SEQ ID NO: 7; CLZ_33, SEQ ID NO: 8; CLZ_34, SEQ ID NO: 9; CLZ_37, SEQ ID NO: 10; CLZ_38, SEQ ID NO: 11; CLZ_40, SEQ ID NO: 12, CLZ_6, SEQ ID NO: 14; CLZ_16, SEQ ID NO: 15; CLZ_22, SEQ ID NO: 16; CLZ_32, SEQ ID NO: 17; CLZ_36, SEQ ID NO: 18; CLZ_42, SEQ ID NO: 19; CLZ_18, SEQ ID NO: 57; CLZ_43, SEQ ID NO: 58; CLZ_44, SEQ ID NO: 59; CLZ_47, SEQ ID NO: 60; CLZ_48, SEQ ID NO: 61; CLZ_49, SEQ ID NO: 62; CLZ_50, SEQ ID NO: 63; CLZ_51, SEQ ID NO: 64; CLZ_52, SEQ ID NO: 65; CLZ_56, SEQ ID NO: 67; CLZ_57, SEQ ID NO: 68; CLZ_60, SEQ ID NO: 69, and CLZ_64, SEQ ID NO: 70). Table 3 contains primers generated from each of the cloned DSTs used in such studies.

Direct Sequencing of TOGA Generated PCR products and Verification by Extended TOGA Method

In other cases, the TOGA PCR product was sequenced using a modification of a direct sequencing methodology (Innis et al., *Proc. Nat'l. Acad. Sci.*, 85: 9436-9440 (1988)).

PCR products corresponding to DSTs were gel purified and PCR amplified again to incorporate sequencing primers at 5' and 3' ends. The sequence addition was accomplished through 5' and 3' ds-primers containing M13 sequencing primer sequences (M13 forward and M13 reverse respectively) at their 5' ends, followed by a linker

sequence and a sequence complementary to the DST ends. Using the Clontech Taq Start antibody system, a master mix containing all components except the gel purified PCR product template was prepared, which contained sterile H_2O, 10X PCR II buffer, 10mM dNTP, 25 mM $MgCl_2$, AmpliTaq/Antibody mix (1.1 μg/μl Taq antibody, 5 U/μl AmpliTaq), 100 ng/μl of 5' ds-primer (5' TCC CAG TCA CGA CGT TGT AAA ACG ACG GCT CAT ATG AAT TAG GTG ACC GAC GGT ATC GG 3', SEQ ID NO: 89), and 100 ng/μl of 3' ds-primer (5' CAG CGG ATA ACA ATT TCA CAC AGG GAG CTC CAC CGC GGT GGC GGC C 3', SEQ ID NO: 90). After addition of the PCR template, PCR was performed using the following program: 94°C, 4 minutes and 25 cycles of 94°C, 20 seconds; 65°C, 20 seconds; 72°C, 20 seconds; and 72°C 4 minutes. The resulting amplified adapted PCR product was gel purified as described above.

The purified ds-extended PCR product was sequenced using a standard protocol for ABI 3700 sequencing. Briefly, triplicate reactions in forward and reverse orientation (6 total reactions) were prepared, each reaction containing 5 μl of gel purified ds-extended N5 PCR product as template. In addition, the sequencing reactions contained 2 μl 2.5X sequencing buffer, 2 μl Big Dye Terminator mix, 1 μl of either the 5' sequencing primer (5' CCC AGT CAC GAC GTT GTA AAA CG 3', SEQ ID NO: 91), or the 3' sequencing primer (5' TTT TTT TTT TTT TTT TTT V 3', where V=A, C, or G, SEQ ID NO: 92) in a total volume of 10 μl.

In an alternate embodiment, the 3' sequencing primer was the sequence 5' GGT GGC GGC CGC AGG AAT TTT TTT TTT TTT TTT TT 3', (SEQ ID NO: 93). PCR was performed using the following thermal cycling program: 96°C, 2 minutes and 29 cycles of 96°C, 15 seconds; 50°C, 15 seconds; 60°C, 4 minutes.

The sequences for (CLZ_62, SEQ ID NO: 71 and CLZ_65, SEQ ID NO: 72) were determined by this method. Table 2 contains the database matches for the sequences determined by this method.

In order to verify that the sequences determined by direct sequencing derive from the PCR product of interest, PCR primers were designed based on the sequences determined by direct sequencing, and PCR reactions were performed using the N1 TOGA PCR reaction products as substrate, as described above for the sequences cloned into the TOPO vector. In short, oligonucleotides were synthesized with the sequence G-A-T-C-G-A-A-T-C extended at the 3' end with a partial MspI site (C-G-G) and an additional 18 nucleotides adjacent to the partial MspI site from the sequence determined by direct sequencing. The 5' PCR primers were paired with the fluorescent labeled universal 3' PCR primer (SEQ ID NO: 23) in PCR reactions with the N1 TOGA PCR reaction product as template. The lengths of these PCR products were compared to the length of the PCR products of interest. Table 3 contains the sequences of the primers used in these studies.

Verification of a Candidate Match Using Extended TOGA Method

In four cases, CLZ_17, (SEQ ID NO: 49); CLZ_26, (SEQ ID NO: 50); CLZ_28, (SEQ ID NO: 51); and CLZ_ 58 (SEQ ID NO: 52) the sequences listed for the TOGA PCR products were derived from candidate matches to sequences present in available Genbank, EST, or proprietary databases. Table 4 lists the candidate matches for each by accession number of the Genbank entry or by the accession numbers of a set of computer-assembled ESTs used to create a consensus sequence.

To determine whether the TOGA PCR products of interest were derived from the sequence of the candidate match, PCR primers were designed with the sequence G-A-T-C-G-A-A-T-C extended at the 3' end with a partial MspI site (C-G-G), and an additional 18 nucleotides adjacent to the terminal MspI site in the candidate match sequence. Each extended primer is combined with the fluorescent labeled universal 3' PCR primer (SEQ ID NO: 23) in a PCR reaction with the product of the first TOGA PCR reaction (N1 reaction products) as the template. The PCR products obtained using an extended primer and the universal 3' primer were compared to products obtained using the original TOGA PCR primers. Primers designed for such studies are shown in Table 4 along with the accession numbers of sequences used to derive the primer sequences.

EXAMPLE 2
Characterization of CLZ 5 (apoD)

Another example of TOGA analysis is shown in Figure 3. In Figure 3, a peak at about 201 is indicated, identified by digital address MspI CACC 201 when a 5' PCR primer (SEQ ID NO: 25) was paired with SEQ ID NO: 23 to produce the panel of PCR products. The PCR product was cloned and sequenced as described in Example 1. To verify the identity of the isolated clone (SEQ ID NO: 2), oligonucleotides were synthesized corresponding to the 5' PCR primer in the second PCR step for each candidate extended at the 3' end with an additional 12-15 nucleotides from the cloned sequence. In this case the 5' PCR primer was G-A-T-C-G-A-A-T-C-C-G-G-C-A-C-C-T-A-C-T-G-G-A-T-C-C-T-G-G (SEQ ID NO: 29). This 5' PCR primer were paired with the fluorescently labeled 3' PCR primer (SEQ ID NO: 23) in PCRs using the cDNA produced in the first PCR reaction as substrate.

As shown in Tables 2 and 3, the CLZ_5 clone (CACC 201) described above corresponds with GenBank sequence X82648, which is identified as a mouse apolipoprotein D (apoD) sequence. Other corresponding apoD GenBank sequences include L39123 (mouse), X55572 (rat), NM_001647 (human). Northern Blot analyses were performed to determine the effect of clozapine and haloperidol on apoD expression in mouse striatum/nucleus accumbens. Also, *in situ* hybridization analyses were performed to determine the pattern of apoD expression in control and clozapine-treated mouse striatum/nucleus accumbens.

Male C57Bl/6J mice (20-28 g) were housed in groups of four on a standard 12/12 hour light-dark cycle with ad libitum access to standard laboratory chow and tap water. The same experimental paradigm used in Example 1 was used for the Northern Blot analyses. Briefly, the control group mice received a single injection of sterile saline (0.1 ml volume), or no injection, and were sacrificed after 45 minutes. The mice subjected to acute neuroleptic treatment were given a single intraperitoneal injection of the typical neuroleptic, haloperidol, (4 mg/kg) or the atypical neuroleptic, clozapine (7.5 mg/kg) and

sacrificed after 45 minutes or 7 hours, as described in Example 1. The mice subjected to chronic neuroleptic treatment received daily subcutaneous injections of haloperidol (4 mg/kg) for 10 days or 14 days, or received daily injections of clozapine (7.5 mg/kg) for 5 days, 12 days or 14 days. All animals were sacrificed in their cages with CO_2 at the indicated times. Brains were rapidly removed and placed on ice. The striatum, including the nucleus accumbens, were dissected out and placed in ice-cold phosphate-buffered saline. The cytoplasmic RNA was isolated by phenol:chloroform extraction of the homogenized tissue according to the method described in Schibler et al., *J. Mol. Bio.*, 142, 93-116 (1980). Poly A enriched mRNA was prepared from cytoplasmic RNA using well-known methods of oligo dT chromatography.

Shown in Fig. 4, Northern Blot analysis was performed using 2 μg poly A enriched mRNA extracted from the striatum/nucleus accumbens of control mice and clozapine-treated mice. The mRNA transcripts were fractionated by electrophoresis on a 1.5% agarose gel containing formaldehyde, transferred to a biotrans membrane by the method of Thomas (Thomas, P. S., *Proc. Natl. Acad. Sci.*, 77,5201-5215 (1980)), and prehybridized for 30 minutes in Expresshyb (Clonetech). A 160 bp insert of CLZ_5 (25-100 ng) was labeled with [α-^{32}P]-d CTP by oligonucleotide labeling to specific activities of approximately $5x10^8$ cpm/μg, added to the prehybridization solution and incubated for 1 hour. Filters were washed to high stringency (0.2 X SSC) (1 X SSC: 0.015 M NaCl and 0.0015 M Na citrate) at 68°C then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) for up to 1 week. Densitrometry analysis on Northern blots was performed by ImageQuant software.

As can be seen in Fig. 4, a 900 bp mRNA was detected in control and clozapine-treated mice which corresponds with the apoD gene. The apoD mRNA expression is progressively up-regulated with clozapine treatment over the two-week time course. It is possible that clozapine may mediate its antipsychotic effect through the regulation of apoD. Alternatively, apoD may be co-regulated by clozapine, in parallel with the mechanism of the clozapine therapeutic effects, and can serve as an indicator of clozapine bioactive levels.

Shown in Fig. 5, Northern Blot analysis was performed using mRNA extracted from the striatum/nucleus accumbens of control mice and haloperidol-treated mice using the above-described method and the same ^{32}P-radiolabelled probe. A 900 bp mRNA was detected in control and haloperidol-treated mice which corresponds with the apoD gene. Interestingly, apoD mRNA expression is slightly down-regulated with acute and chronic haloperidol treatment. These results reveal that clozapine and haloperidol have a differential effect on apoD expression.

Figure 6 is a graphical representation comparing the results of the clozapine treatment TOGA analysis of clone CLZ_5 (CACC 201) shown in Fig. 4 and the clozapine treatment Northern Blot analysis of clone CLZ_5 shown in Figure 5. The Northern Blot was imaged using a phosphoimager to determine the amount of apoD mRNA in each clozapine-treated sample relative to the amount of mRNA in the control sample. As can be seen, the clozapine treatment TOGA analysis shows correlation with the clozapine treatment Northern Blot analysis.

Figure 7A-C shows an *in situ* hybridization analysis, demonstrating the apoD expression in mouse brain. The *in situ* hybridization was performed on free-floating sections (25 μM thick) as described (Thomas et al., *J. Neurosci. Res.*, 52, 118-124 (1998)). Coronal sections were hybridized at 55°C for 16 hours with an ^{35}S-labeled, single-stranded 160 bp antisense cRNA probe of CLZ_5 at 10^7 cpm/ml. The probe was synthesized from the 3'-ended cDNA TOGA clone CLZ-5 using the Maxiscript Transcription Kit (Ambion, Austin, TX). Excess probe was removed by washing with 2 X SSC (I X SSC = 0.015 M NaCl/0.0015 M Na citrate) containing 14 mM β-mercaptoethanol (30 minutes), followed by incubation with 4 μg/ml ribonuclease in 0.5 M NaCl/0.05 M EDTA/0.05 M Tris-HCl, pH 7.5, for 1 hour at 37°C. High stringency washes were carried out at 55°C for 2 hours in 0.5 X SSC/50% formamide/0.01 M β-mercaptoethanol, and then at 68°C for 1 hour in 0.1 X SSC/0.01 M β-mercaptoethanol/0.5% sarkosyl. Slices were mounted onto gelatin-coated slides and dehydrated with ethanol and chloroform before autoradiography. Slides were exposed

for 1-4 days on Kodak X-AR film and then dipped in Ilford K-5 emulsion. After 4 weeks, slides were developed with Kodak D19 developer, fixed, and counterstained with Richardson's blue stain.

Fig. 7A shows CLZ-5 (apoD) mRNA expression in mouse anterior brain, 7B shows apoD mRNA expression in midbrain and 7C shows apoD expression in posterior brain. In all brain sections apoD is expressed by astroglial cells, pial cells, perivascular fibroblasts and scattered neurons. This is consistent with previous studies examining the expression of apoD in mice, rabbits and humans (Yoshida et al., *DNA and Cell Biology*, 15, 873-882 (1996); Provost et al., *J. Lipid Res.*, 32, 1959-1970 (1991); Navarro et al., *Neurosci. Lett.*, 254, 17-20 (1998).

The Northern blot results (Figures 4 and 6) indicated that apoD was induced by clozapine in the striatum of mouse brain. To investigate additional sites of apoD induction, *in situ* hybridization analysis was performed on brains from saline- and clozapine-treated mice. Figure 8A-I presents an *in situ* hybridization analysis, showing clone CLZ_5 (apoD) mRNA expression in mouse anterior (8A-C), mid (8D-F), and posterior (8G-I) brain following saline treatment (top row) or clozapine treatment (7.5 mg/kg) for 5 days (middle row) and 14 days (bottom row), using previously described methods. Animals were sacrificed by intracardial perfusion with 4% paraformaldehyde and the brains removed, post-fixed for 12 hours, cryoprotected with 30% sucrose and rapidly frozen at -70°C. At low magnification, increases in apoD mRNA were observed at both five days and two weeks of clozapine treatment in the striatum, cortex, globus pallidus (GP), and thalamus. Increases in apoD expression were also detected in white matter tracts, predominantly the corpus callosum (cc), anterior commissure, internal capsule (ic) and optic tract (opt). At high magnification, it was evident that the increased apoD hybridization signal in the striatum, globus pallidus, and thalamus of the drug-treated animals was primarily due to an increase in the number of cells expressing detectable apoD, although some cells with higher apoD expression were also observed.

Using a monoclonal antibody directed against full-length apoD, immunohistochemistry analyses were performed to evaluate changes in apoD protein expression in response to clozapine. Increase in protein expression correlated well with increases in mRNA expression (data not shown). Combined *in situ* hybridization and immunohistochemical studies demonstrated that increases in apoD levels were localized primarily to neurons and astrocytes of the striatum and oligodendrocytes in various white matter tracts throughout the brain.

Figure 9A-H shows a darkfield photomicrograph demonstrating upregulated apoD mRNA expression in various brain regions, including the corpus callosum (cc, Fig. 9A, E); caudate putamen (CPu, Fig. 9B, 7F); anterior commissure (aca, Fig. 9C, 9G); and globus pallidus (GP, Fig. 9D, 9H). *In situ* hybridizations were perfomed as described above, using an antisense ^{35}S-labeled apoD riboprobe on brains from control (Fig. 9A-D) and clozapine-treated (Fig. 9E-H) animals. The observed upregulation of apoD was due to an increase in the amount of apoD expressed per cell.

Figure 10A, B shows a darkfield photomicrograph demonstrating upregulated apoD mRNA expression in the internal capsule (ic). Figure 10C, D shows a brightfield view of the optic tract (opt) demonstrating upregulation of apoD expression in oligodendrocytes. *In situ* hybridizations were perfomed as described above, using an antisense ^{35}S-labeled apoD riboprobe on brains from control (10A, C) and clozapine-treated (10B, D) animals. As shown in Fig. 10D, the cells prominantly expressing apoD in the optic tract have a box-like morphology and are lined up in a serial array, presumably along axonal tracts. Such features are characteristic of oligodendrocytes, which synthesize the insulating myelin coating of nerve fibers. *In situ* hybridization experiments performed on brains from haloperidol-treated mice did not reveal substantial increases in apoD expression in gray or white matter regions (data not shown).

White matter tracts comprise nerve fiber bundles connecting different regions of the brain. The predominant cells in these regions are astrocytes and oligodendrocytes, both of which have been shown to express apoD (Boyles et al., *J Lipid Res* 31:2243-2256

(1990); Navarro et al., *Neurosci Lett* 254:17-20 (1995); Provost et al., *J Lipid Res* 32 (1991)). To determine which cell types are responsible for the increase in apoD signal, co-localization studies were performed using a ^{35}S-labeled apoD riboprobe in combination with either an antibody specific for an astrocyte marker, glial fibrillary acidic protein (GFAP), or an antibody specific for an oligodendrocyte marker, 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNP) (Boehringer Mannheim, Germany). The immunoreaction was detected with Vectastain ABC ™ kit (Vector Laboratory, Inc., Burlingame, CA) according to the manufacturer's instructions.

Free floating brain sections were incubated with blocking solution (4% bovine serum albumin in 0.1% Triton X-100/PBS) for 2 hours at room temperature, followed by incubation with anti-GFAP or anti-CNP antiserum (dilution 1:500) in blocking solution for 16-20 hours at 4°C. Sections were then washed with 0.1% Triton X-100/PBS and incubated with secondary biotinylated antibody (1:200 dilution in blocking solution) for 2 hours at room temperature. The sections were then washed with 0.1% Triton X-100/PBS, incubated for 1 hour with ABC reagent (1:1 in blocking solution) and finally washed with 0.1% Triton X-100/PBS. Enzymatic development was performed in 0.05% diaminobenzene in PBS containing 0.003% hydrogen peroxide for 3-5 minutes.

Fig. 11 shows sections of striatum and optic tract in control and clozapine-treated animals. Thick arrows indicate the co-localization of GFAP and apoD, while thin arrows indicate the expression of apoD alone. Fig. 11A, B shows that in untreated striatum, many GFAP-positive cells in both gray and white matter regions are positive for apoD. Fig. 11D, E shows that in brain from clozapine-treated animals, an increase in the amount of apoD was observed in a small subset of GFAP-positive cells in the striatum. Additionally, there was an increase in the number of non-GFAP-positive cells expressing apoD in the striatum, as well as the globus pallidus and thalamus which are presumptively neurons, based on size and morphology.

Fig. 11C, F shows GFAP and apoD co-localization in the optic tract in control (11C) and clozapine-treated (11F) animals. While some astrocytes express apoD mRNA,

the cells responsible for the predominant apoD transcript upregulation did not label with GFAP and thus are likely oligodendrocytes. In other white matter regions, such as the corpus callosum, anterior commissure and internal capsule, the non-GFAP expressing cells that express apoD are likely to be oligodendrocytes as well, although expression in microglia can not be ruled out. Fig. 11G, H shows apoD immunohistochemistry with an anti-human apoD primary antibody (Novocastra, Newcastle, UK) in the optic tract of control saline (11G) and clozapine-treated animals (11H).

Co-localization studies performed using anti-CNP antibody showed CNP immunoreactivity in white matter tracts throughout the CNS which correlated with areas of apoD mRNA hybridization signals, indicating the expression of apoD in oligodendrocytes. However, within the gray matter regions of the striatum, there was no co-localization consistent with the neuronal accumulation of apoD (data not shown).

Figure 12 shows a Northern Blot analysis of clone CLZ_5 expression in cultured glial cells treated with clozapine (100 nM and 1 μM) for 1 day or 7 days. Glial cell cultures were produced from postnatal (day 2) rats. The cells were treated with different concentrations of clozapine for different lengths of time before mRNA extraction as follows: A= control (no clozapine), B= 100 nM clozapine, 1 day, C= 1μM clozapine, 1 day, D= 100 nM clozapine, 1 week, E= 1μM clozapine, 1 week. 20 μg of total cytoplasmic RNA from glial cell cultures were electrrophoresed on a 1.5% agarose gel containing formaldehyde, blotted, and probed as previously described. Interestingly, apoD mRNA levels were down-regulated in mixed glial cell cultures treated with clozapine (both 100 nM and 1 μM) for 1 week, suggesting that perhaps neurons and glia display different mechanisms for apoD regulation.

TOGA methodology, Northern blot analyses, and *in situ* hybridization studies have demonstrated an increase in apoD mRNA expression in both white and gray matter regions of mouse brain in response to chronic clozapine administration. Colocalization studies, combining *in situ* hybridization and imunohistochemistry methods have revealed that apoD mRNA levels are increased in both neurons and glial cells with clozapine

administration. The evidence indicates that the glial cells responsible for the most dramatic increases in apoD expression are primarily oligodendrocytes, but a subset of astrocytes also have increased apoD expression after clozapine treatment. In contrast, TOGA, Northern blot and *in situ* hybridization analyses showed that apoD expression was not affected by haloperidol treatment.

In addition to the mouse studies described above which show that apoD is regulated by chronic antipsychotic drug administration, studies using schizophrenic and bipolar human subjects showed that apoD expression is increased in the prefrontal cortex of such patients. The combined results suggest that apoD is a marker for neuropathology associated with psychiatric disorders and therefore can be used to target abnormalities in specific anatomical brain regions.

ApoD was initially identified as a constituent of plasma high-density lipoproteins (HDLs), which also contain phospholipids, cholesterol and fatty acids (McConathy et al., *Fed. Eur. Biochem. Soc. Lett.*, 37: 178 (1973)). In the blood, apoD is thought to play a role in reverse cholesterol transport, the removal of excess cholesterol from tissues to the liver for catabolism (Oram et al., *J. Lipid. Res.*, 37: (1996)). In addition to abundant expression in human serum, apoD is major protein component in cyst fluid from women with human breast cystic disease (Balbin et al., *Biochem. J.*, 271: 803 (1990)) and also is widely expressed in numerous tissues, including liver, kidney, intestine, spleen and brain (Drayna et al., *J. Biol. Chem.*, 261: (1986)). In the CNS of humans, as in other species (Provost et al., *J. Lipid Res.*, 32: (1991); Seguin et al., *Mol. Brain Res.*, 30: 242 (1995); Smith et al., *J. Lipid Res.*, 31: 995 (1990)), apoD is expressed primarily in glial cells, pial cells, perivascular cells, and some neuronal populations (Navarro et al., *Neurosci. Lett.*, 254: 17 (1995); Kalman et al., *Neurol. Res.*, 22: 330 (2000)). The physiological role for apoD within the CNS is not known, however, it has been shown to bind several hydrophobic ligands, including sterols and steroid hormones (Dilley et al., *Breast Canc. Res. Treat.*, 16: 253 (1990); Lea, O. A., *Steroids*, 52: 337 (1988)) suggesting a role in extracellular lipid transport in the brain. ApoD has also been shown to bind arachidonic acid Morais-Cabral et al., *FEBS Lett.*, 366: 53 (1995)) implicating it in functions

associated with cell membrane remodeling and prostaglandin synthesis. In the regenerating sciatic nerve, a process that involves massive membrane degradation and lipid release, apoD concentrations are increased 500-fold (Boyles et al., *J. Biol. Chem.*, 265: 17805 (1990)). Recent reports have also demonstrated an increase in apoD expression in rat brain after experimental and chemical lesioning of the entorhinal cortex and hippocampus, respectively (Ong et al., *Neurosci.*, 79:359 (1997); Terisse et al., *Mol. Brain Res.*,70: 26 (1999)). Additionally, in humans, apoD accumulates in the cerebrospinal fluid and hippocampi of patients with Alzheimer's, and other neurological diseases (Terisse et al., *J. Neurochem.*, 71: 1643 (1998)). Hence, apoD may be functioning during pathological situations or its expression may represent an effort to compensate for neuropathology associated with such insults.

The pattern of apoD expression in the brain suggests that apoD may play an important role in psychotic disease. It is widely believed that imbalances in basal ganglia circuitry contribute to psychotic behaviors and that blockade of specific receptors in these regions is responsible for neuroleptic action. The neuronal increases in apoD mRNA expression observed in neurons of the striatum and globus pallidus are consistent with this hypothesis.

In addition, the apoD induction observed in the internal capsule is of particular interest. The internal capsule consists of massive nerve fibers connecting the thalamus to the cortex and is an area of convergence for the fiber tracts running transversely through the striatum. The thalamus is a relay station for virtually all information passing to the cortex and coordinated cortico-thalamic activity is essential for normal consciousness. Recent theories have associated psychotic behavior with disruptions in cortico-thalamic oscillations. An upregulation of apoD expression in the internal capsule may play a role in restoring the proper balance of neuronal communication.

In addition, abnormal lipid neurochemistry resulting from abnormal lipid transport or metabolism has been associated with psychotic disease, such as schizophrenia (Walker et al., *Br. J. Psych.*, 174, 101-104 (1999)). Relating impaired

cholesterol metabolism with psychotic disease, a number of reports have described psychoses as an initial manifestation of Neimann-Pick Disease, type C (Campo, et al., *Develop. Med. and Child Neurol.*, 40, 126-129 (1998); Shulman, et al., *Neurology*, 45, 1739-1743 (1995); Turpin, et al., Dev. Neurosci., 13, 304-306 (1991)), which is an autosomal recessive disease associated with abnormal cholesterol metabolism (Yoshida et al., *DNA and Cell Biology*, 15, 873-882 (1996)). Further reports have suggested that myelin dysfunction may cause mental illness. Given that the majority of cholesterol in the brain is incorporated into myelin, abnormal cholesterol metabolism may result in myelin dysfunction. Myelin acts as an insulator along nerve axons allowing for the rapid propagation of action potentials along nerve fibers. Molecular abnormalities of myelin may result in the dysregulated neural connectivity that has been hypothesized to be causative in mental illnesses (Weickert, et al., *Schizophrenia Bull.*, 24, 303-316 (1998)).

While the physiological function of apoD in the CNS is not clear, several lines of evidence suggest a role for apoD as a vehicle for extracellular lipid transport and lipid movement, particularly cholesterol, in the nervous system. ApoD is a constituent of plasma high-density lipoproteins (HDLs), which also contain phospholipids, cholesterol and fatty acids. While not much is known about HDL compared to the other plasma lipoproteins, LDL and VLDL, it is widely believed that HDLs protect against cardiovascular disease by removing excess cholesterol from cells of arterial walls. This removal involves the direct interaction of HDL lipoproteins with plasma membrane domains and subsequent transport to the liver for catabolism (Oram, et al., *J. Lipid Res.*, 37, 2473-2491 (1996)). Additionally, apoD is synthesized and secreted by cultured astrocytes, which secretion has been shown to increase in the presence of cholesterol derivatives (Patel, et al., *Neuroreport* 6, 653-657 (1995)). Further, it has also been demonstrated that apoD levels are increased in Niemann Pick Disease, type C, which is associated with elevated levels of cholesterol. These studies provide evidence of a functionally significant role for apoD in cholesterol transport in the CNS.

In addition to the studies correlating cholesterol levels and psychotic behavior, other studies have found a correlation between cholesterol levels and treatment with

neuroleptics. For example, reports dating back to 1960 have demonstrated an increase in the serum cholesterol of patients treated with conventional neuroleptics, such as chlorpromazine and haloperidol (Spivak et al., *Clin. Neuropharm.*, 22, 98-101 (1999). Fleischhacker et al., *Pharmacopsychiatry*, 19, 111-114 (1986); Clark et al., *Clin. Pharm. and Therapeutics*, 11, 883-889 (1970)). However similar increases are not observed with the newer, atypical antipsychotics, such as fluperlapine and clozapine (Spivak et al., *Clin. Neuropharm.*, 22, 98-101 (1999). Fleischhacker et al., *Pharmacopsychiatry*, 19, 111-114 (1986); Boston, et al., *Biol. Psych.*, 40, 542-543 (1996)). Interestingly, the present results reveal that clozapine and haloperidol have a differential effect on apoD expression, which may account for the observed differences in cholesterol regulation. While the mechanism for these cholesterol changes is not known, the present data suggest that neuroleptic-induced changes in apoD expression combined with the ability of apoD to bind cholesterol may provide an explanation for the neuroleptic-induced changes in cholesterol levels.

In addition to studies relating to cholesterol movement, reports have focused on the link between disrupted phospholipid and fatty acid metabolism and psychiatric disorders (for a review see Horrobin, et al., *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 60, 141-167 (1999)). For example, numerous studies have reported differences in levels of total membrane phospholipid content, fatty acid levels, cholesterol levels and cholesteryl esters in fibroblasts and/or frontal cortex of schizophrenics (Keshavan et al., *J Psychiatry Res.*, 49, 89-95 (1993); Mahadik et al., *Schizophrenia Res.* 13, 239-247 (1994); Sengupta et al., *Biochem. Med.*, 25, 267-275 (1981); Stevens, *Schizophr. Bull., 6, 60-61 (1972)*). Membrane phospholipids act as precursors in numerous signaling systems (e.g., inositol phosphates, arachidonic acid, platelet activation factors, and eicosaniods) and comprise the membrane environment for neurotransmitter-mediated signal transduction. Thus, altered membrane phospholipid metabolism could have significant consequences for neuronal communication, resulting in behavioral abnormalities.

Alterations in plasma membrane structure and function may result from the altered content and distribution of membrane lipids and fatty acids, such as arachidonic acid. Arachidonic acid is released by the action of numerous phospholipase enzymes, primarily phospholipase A2, and is a substrate for prostglandins and leukotriene synthesis. While the molecular mechanisms underlying abnormalities in the complex system of phospolipid biochemistry are not known, several groups have demonstrated an increase in phospholipase A2 activity in the plasma and brains of schizophrenic patients (Gattaz et al., *Biol. Psychiatry.*, 22, 421-426 (1987); Ross et al., *Arch. Gen. Psychiatry.*, 54, 487-494 (1997); Ross et al., *Brain Research,* 821, 407-413 (1999)). In addition, plasma phospholipase A2 levels have been shown to be decreased after neuroleptic therapy (Gattaz et al., *Biol. Psychiatry,* 22, 421-426 (1987)). Other molecular candidates implicated in psychotic disease include phospholipase C enzymes, diacyl glycerol kinases, and inositol phosphates (Horrobin et al., *Prostaglandins, Leukotrienes and Essential Fatty Acids, 60, 141-167 (1999)*).

Interestingly, in addition to binding cholesterol, apoD has been shown to specifically bind arachidonic acid. ApoD is an atypical apolipoprotein in that it does not share sequence homology with other apolipoproteins (Weech et al., *Prog. Lipid Res.*, 30, 259-266 (1991)) but, rather, is a member of the lipocalin superfamily of proteins, which function in the transport of small hydrophobic molecules, including sterols, steroid hormones, and arachidonic acid (Balbin et al., *Biochem. J.*, 271, 803-807 (1990); Dilley et al., *Breast Cancer Res. Treat.*, 16, 253-260 (1990); Lea, *Steroids,* 52, 337-338 (1988); Boyles et al., *J. Lipid Res.*, 31, 2243-2256 (1990)). As a lipid binding protein, apoD can affect fatty acid composition, cholesterol levels and membrane phospholipids, all of which will affect plasma membrane composition and structure. Also, since apoD specifically binds cholesterol, arachidonic acid and other lipids, alterations in the levels of apoD can affect lipid metabolism and signal transduction by affecting substrate availability for these pathways.

Further implicating the role of apoD in psychosis is the observation that apoD may have a chromosomal linkage with schizophrenia. The chromosomal location of

apoD is 3q26. Genetic studies have implicated a potential association between schizophrenia and chromosome 3q, however the linkage is relatively inconsistent (reviewed by Maier, et al., *Curr. Opin. Psych.*, 11, 19-25 (1998)).

Northern blot analysis on striata from haloperidol-treated mice did not reveal similar increases in apoD expression as clozapine. Schizophrenia is a heterogeneous disorder encompassing many subtypes. The observed differences in clinical efficacy between clozapine and haloperidol may reflect different subtypes of schizophrenia that are associated with different pathways or mechanisms. Thus, regulation of apoD may represent a unique mechanism of action for clozapine.

In this regard, a serotonin sub-type such as $5HT_{2a}$ and $5HT_{2c}$ may provide a pharmacological mechanism for clozapine's effect on apoD expression. Preliminary results demonstrate that treatment with ketanserin and mesulergine, $5HT_{2a/2c}$ and $5HT_{2c}$-selective antagonists respectively, results in an apparent upregulation of apoD mRNA expression in mouse brain. It is known that the striatum expresses a number of 5HT receptor subtypes, including the $5HT_{2c}$, which subtype may mediate clozapine's effect on apoD expression. In contrast, cultured glial cells or astrocytes do not appear to express $5HT_{2c}$ receptors. Thus the downregulation observed in these cells may reflect actions at a different 5HT subtype, such as $5HT_{2a}$, or a different receptor. Additionally, in hypertension studies, ketanserin has been associated with a decrease in total cholesterol levels and an upregulation of another apolipoprotein, apo A1 (Loschiavo, et al., *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 28, 455-457 (1990)). The similar effects observed by both ketanserin and clozapine suggest that they may be working through the same receptor subtype(s).

The finding that apoD mRNA levels are increased by clozapine links apolipoproteins and the mechanism of action of neuroleptic drugs. The proposed role of apoD in CNS lipid transport, combined with the recent evidence that schizophrenia and other neuropsychiatric illnesses are accompanied by abnormalities in lipid metabolism, suggest that apoD could play an important role in the action of clozapine.

EXAMPLE 3

Characterization of CLZ 40

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the various analyses described below. Briefly, in the clozapine studies, the control group mice received a single injection of sterile saline (0.1 ml volume), or no injection, and were sacrificed after 45 minutes. The mice subjected to acute clozapine treatment were given a single intraperitoneal injection of clozapine (7.5 mg/kg) and sacrificed after 45 minutes or 7 hours, as described in Example 1. The mice subjected to chronic clozapine treatment received daily subcutaneous injections of clozapine (7.5 mg/kg) for 5 days, 12 days or 14 days. All animals were sacrificed in their cages with CO_2 at the indicated times. Brains were rapidly removed and placed on ice. The striatum, including the nucleus accumbens, were dissected out and placed in ice-cold phosphate-buffered saline. The mRNA was prepared according to the method described in Example 2.

For the morphine studies, male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1 and divided into the following groups:

1) a control group, in which the mice were subcutaneusly implanted with one placebo pellet upon halothane anaesthesia;

2) an acute morphine group, in which the mice received a morphine intraperitoneal injection of 10 mg/kg;

3) a chronic or tolerant group, in which mice were rendered drug-tolerant and dependent by means of subcutaneous implantation of a single pellet containing 75 mg of morphine free base for 3 days; and

4) a withdrawal group, in which the mice rendered tolerant to morphine were injected intraperitoneally with naltrexone 1 mg/kg. Animals were sacrificed in their cages with CO_2 at 72 hours after placebo or morphine pellet implantation, or 4 hours after single injection of morphine, or 4 hours after administration of naltrexone to morphine-tolerant mice. Their brains were rapidly removed. The striatum, including the nucleus accumbens,

and block of tissues containing the amygdala complex were dissected under microscope and collected in ice-cold RNA extraction buffer.

The TOGA data shown in Figures 13 and 14 were generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-T-G-T; SEQ ID NO: 26) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

The results of TOGA analysis using a 5' PCR primer with parsing bases C-G-A-C-G-G-T-A-T-C-G-G-T-T-G-T (SEQ ID NO: 26) are shown in Figs. 13 and 14, which show PCR products produced from mRNA isolated from the striatum/nucleus accumbens of mice treated with clozapine (Fig. 13) or morphine (Fig. 14). In Fig. 13, the vertical index line indicates a PCR product of about 266 b.p. that is present in control cells, and whose expression decreases in the striatum/nucleus accumbens of mice treated with clozapine for 45 minutes, 7 hours, 5 days, 12 days, and 14 days. The down-regulation of CLZ_40 occurs as early as 45 minutes following clozapine treatment and remains downregulated for at least 14 days.

In Fig. 14, the vertical index line indicates a PCR product of about 266 b.p. that is present in control cells, and whose expression differentially regulated in control striatum (PS), acutely treated striatum (AS), withdrawal striatum (WS), control amygdala (PA), acutely treated amygdala (AA), chronically treated amygdala (TA), and withdrawal amygdala (WA). The expression of CLZ_40 product is greater in striatum than in amygdala. Further, CLZ_40 displays chronic-specific or withdrawal-specific regulation in both of these brain regions. In striatum, CLZ_40 is downregulated in withdrawal striatum but not acutely treated striatum. In amygdala, CLZ_40 is slightly upregulated in acutely treated amygdala and increasingly upregulated in chronically treated amygdala and withdrawal amygdala.

Shown in Fig. 15, Northern Blot analysis was performed using mRNA extracted from the striatum/nucleus accumbens of control mice and clozapine-treated mice. Briefly, an agarose gel containing 2μg of poly A enriched mRNA as well as size standards was electrophoresed on a 1.5% agarose gel containing formaldehyde, transferred to a biotrans membrane, and prehybridized for 30 minutes in Expresshyb (Clonetech). An 265 bp insert of CLZ_40 (25-100 ng) was labeled with [α-^{32}P]-d CTP by oligonucleotide labeling to specific activities of approximately $5x10^8$ cpm/μg and added to the prehybridization solution and incubated 1 hour. Filters were washed to high stringency (0.2 X SSC) (1 X SSC: 0.015 M NaCl and 0.0015 M Na citrate) at 68°C then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) for up to 1 week. As shown in Fig. 15, a 9-12 Kb transcript was detected in control and clozapine-treated mice which decreases dramatically after 45 minutes with clozapine treatment and remains down-regulated for at least 14 days.

Figure 16 is a graphical representation comparing the results of the clozapine treatment TOGA analysis of clone CLZ_40 shown in Fig. 13 and the clozapine treatment Northern Blot analysis of clone CLZ_40 shown in Figure 15. The Northern Blot was imaged using a phosphoimager to determine the amount of CLZ_40 mRNA in each clozapine-treated sample relative to the amount of mRNA in the control sample. As can be seen, the clozapine treatment TOGA analysis shows correlation with the clozapine treatment Northern Blot analysis.

Figure 17A-B is an *in situ* hybridization analysis, demonstrating CLZ_40 mRNA expression in the mouse brain. *In situ* hybridization was performed on free-floating sections (25 μM thick). Coronal sections were hybridized at 55°C for 16 hour with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_40 at 10^7 cpm/ml. The probe was synthesized from the 3'-ended cDNA TOGA clone using the Maxiscript Transcription Kit (Ambion, Austin, TX). Excess probe was removed by washing with 2 X SSC (I X SSC = 0.015 M NaCl/0.0015 M Na citrate) containing 14 mM β-mercaptoethanol (30 minutes), followed by incubation with 4 μg/ml ribonuclease in 0.5 M NaCl/0.05 M EDTA/0.05 M Tris-HCl, pH 7.5, for 1 hour at 37°C. High stringency

washes were carried out at 55°C for 2 hours in 0.5 X SSC/50% formamide/0.01 M β-mercaptoethanol, and then at 68°C for 1 hour in 0.1 X SSC/0.01 M β-mercaptoethanol/0.5% sarkosyl. Slices were mounted onto gelatin-coated slides and dehydrated with ethanol and chloroform before autoradiography. Slides were exposed for 1-4 days to Kodak X-AR film and then dipped in Ilford K-5 emulsion. After 4 weeks, slides were developed with Kodak D19 developer, fixed, and counterstained with Richardson's blue stain. Interestingly, CLZ_40 mRNA is specifically expressed in the nucleus accumbens and pyriform cortex (Fig. 17A), and dentate gyrus (Fig. 17B), but is not detected in any other brain regions.

At present, CLZ_40 (SEQ ID NO: 12) is of unknown identity. However, the CLZ_40 DST has been PCR amplified and the extended sequence clone of CLZ_40 (SEQ ID NO: 13) matches an EST in the GenBank database (AI509550) as shown in Table 4. The observation that CLZ_40 is down-regulated with clozapine treatment suggests a potential association with the therapeutic effects of clozapine. Furthermore, its highly unique gene expression pattern is like no other gene identified to date, and its presence in the nucleus accumbens may implicate CLZ_40 in a number of functional roles associated with this structure, namely limbic/mental behavior and addiction.

Addiction to opiates and other drugs of abuse is a chronic disease of the brain, most likely resulting from molecular and cellular adaptations of specific neurons to repeated exposure to opiates (Leshner, A., *Science*, 278, 45-47 (1997)). An important neural substrate implicated in the opioid reinforcement and addiction is the mesolimbic system, notably the nucleus accumbens (Everitt, et al, *Ann. N.Y. Acad. Sci.*, 877, 412-438 (1999)). All highly addictive drugs, such as opiates, cocaine and amphetamines, produce adaptations in the neural circuitry of the nucleus accumbens, but the precise relationships are unknown. The molecular neuroadaptation which takes place in this structure may offer important insight into the mechanisms of drug addiction. CLZ_40 is a likely candidate for involvement in such mechanisms due to its specific expression in the nucleus accumbens. Elucidation of the biology underlying psychoses and addiction is

key to understanding the underlying causes of such disorders and may lead to the development of more effective treatments, including anti-addiction medications.

Furthermore, the behavioral mechanisms associated with addiction reflect mechanisms of learning and memory (White, N., *Addiction*, 91, 921-949 (1996)). The hippocampal system has long been associated with learning and memory, including forms of conditional associative learning (Sziklas, et al., *Hippocampus*, 8, 131-137 (1998)), which is the form of learning associated with addiction (Di Chiara, et al., *Ann. N.Y. Acad. Sci.*, 877, 461-85 (1999)). The expression of CLZ_40 in the hippocampus suggests that this gene may provide a link with such learning processes.

TABLE 1

Seq ID	Clone ID	Digital Address (MspI)		Control	45 minutes	7 Hour	5 Day	12 Day	14 Day
		AAAA	276	314	189	183	299	292	227
		AAAG	91	18	27	34	52	60	35
		AAAG	446	135	127	219	245	529	210
		AAAG	449	135	173	219	245	775	210
		AACA	109	31	17	85	54	51	72
		AACA	117	38	30	45	39	72	118
		AACA	137	355	205	163	129	111	186
		AACA	307	56	58	65	55	134	64
		AACG	375	633	450	420	528	968	1015
		AACG	498	717	221	349	438	1647	1392
		AACT	85	481	139	145	108	281	580
		AACT	112	297	162	391	538	330	555
		AACT	392	176	267	427	303	296	315
		AAGA	309	22	19	42	91	61	36
		AAGA	324	37	15	12	83	31	46
		AAGC	446	284	212	155	249	318	338
		AAGC	498	456	369	309	495	735	862
		AAGG	270	169	191	176	243	283	265
		AAGG	457	191	129	152	228	269	320
		AAGG	497	265	164	208	432	390	512
		AAGT	282	75	73	82	84	204	105
		AATA	90	47	46	39	74	115	65
60	CLZ_47	AATA	136	817	555	589	297	245	397
		AATA	194	70	81	70	133	181	112
		AATC	352	108	108	128	144	631	140
		AATC	499	49	32	43	67	75	67
		AATT	425	38	30	38	37	64	45
		ACAA	80	92	67	109	319	353	110
		ACAA	122	58	95	107	46	818	98
		ACAA	239	117	45	133	49	217	137
		ACAC	145	313	365	296	277	750	631
		ACAC	273	163	169	262	274	800	338
		ACAG	81	167	81	57	137	314	253
		ACAG	270	117	94	117	93	236	213
		ACAG	296	32	34	71	47	89	62
		ACAG	413	39	43	52	43	88	81
		ACAG	437	25	20	41	22	55	41
		ACAT	94	91	151	149	91	340	195
		ACCA	109	318	505	352	289	189	200

		ACCA	418	33	28	46	40	65	39
		ACCA	422	28	23	44	39	51	39
		ACCC	394	32	55	40	38	162	37
		ACCC	493	54	42	57	48	93	69
		ACCG	90	181	155	184	217	382	208
		ACCG	220	169	113	262	189	335	247
		ACCG	489	33	30	28	44	63	41
		ACCT	119	117	121	47	86	300	164
		ACCT	490	78	76	57	120	165	133
		ACGA	77	567	133	109	72	1143	1079
		ACGA	92	61	56	56	76	195	63
		ACGA	292	349	247	165	190	306	148
		ACGC	78	243	31	51	236	2323	1676
		ACGC	118	1026	737	849	292	442	513
		ACGC	210	243	284	293	343	682	735
		ACGC	284	27	50	60	195	159	94
		ACGC	474	50	91	87	107	190	131
		ACGG	264	140	108	117	115	294	172
		ACGG	335	245	104	102	110	131	159
59	CLZ_44	ACGG	352	171	407	428	538	683	553
		ACGG	382	37	53	113	154	141	103
		ACGG	406	114	233	267	217	219	211
		ACTA	88	28	37	33	29	219	41
		ACTA	199	38	84	48	120	365	66
		ACTC	88	64	30	71	124	108	81
		ACTC	105	54	121	172	155	352	294
		ACTG	266	23	35	116	35	87	44
		ACTG	468	148	80	53	74	58	68
		ACTT	436	490	549	450	494	435	504
		AGAA	104	86	210	143	63	39	106
		AGAA	196	62	75	43	85	172	97
		AGAA	462	42	29	25	27	64	42
		AGAC	410	362	307	538	530	918	442
		AGAT	79	41	73	50	64	193	70
		AGAT	251	622	622	746	691	562	696
		AGAT	295	294	252	263	281	303	263
		AGAT	456	603	525	571	639	588	559
		AGCA	177	21	38	46	64	163	100
		AGCC	295	661	444	517	421	360	475
		AGCC	468	112	99	110	165	145	146
		AGCG	202	385	349	433	339	334	334
		AGCT	95	162	963	1168	2493	3990	1420
		AGCT	260	89	78	58	296	86	294

		AGGA	426	365	532	720	670	896	802
		AGGC	104	46	86	169	163	642	339
		AGGG	177	739	251	249	210	174	408
		AGGG	242	165	110	192	222	376	293
		AGGG	492	35	48	33	46	98	75
		AGGG	498	50	47	69	79	155	111
		AGGT	99	55	36	55	80	83	61
		AGGT	103	29	27	31	50	84	38
		AGGT	119	835	719	808	518	466	643
1	CLZ_3	AGTA	106	657	1677	1883	894	832	1282
		AGTC	97	297	229	215	158	111	180
		AGTC	178	519	351	238	263	353	269
		AGTC	410	65	93	107	85	175	156
		AGTG	498	532	851	1476	1209	2196	1092
		AGTT	378	48	33	61	40	68	56
		ATAA	183	428	319	426	353	915	583
		ATAA	225	17	40	39	49	128	82
		ATAG	94	52	98	63	343	469	76
		ATAG	108	1111	995	933	833	713	869
		ATAG	402	495	416	472	546	535	482
		ATAT	140	37	20	44	53	45	57
		ATCA	90	423	666	451	172	379	180
		ATCA	199	774	588	493	335	336	352
		ATCT	99	59	43	56	35	125	67
		ATCT	392	139	176	287	262	569	226
		ATGA	162	91	95	127	239	191	262
		ATGC	78	138	91	111	190	466	148
		ATGC	124	317	884	743	403	164	317
		ATGC	236	15	23	76	7	54	119
		ATGC	344	153	108	131	187	217	185
		ATGG	96	118	231	173	115	113	305
		ATGG	365	15	26	22	25	63	29
		ATGT	378	28	47	90	54	108	80
		ATGT	383	26	61	78	40	136	63
		ATTA	256	36	29	27	46	61	81
		ATTA	259	48	54	55	65	75	106
		ATTG	88	100	147	147	262	318	114
		ATTG	485	22	27	27	26	100	29
		ATTT	186	87	60	58	64	190	122
		ATTT	189	99	79	74	85	209	127
		ATTT	313	79	49	94	86	511	197
		ATTT	499	62	80	78	61	265	114
		CAAA	423	398	255	395	302	506	434

		CAAC	471	87	67	99	85	134	104
		CAAC	474	93	77	109	85	151	128
		CAAT	319	23	18	22	16	66	30
		CACA	253	771	716	598	626	684	579
		CACA	348	847	303	241	181	316	342
		CACA	374	205	116	308	211	262	175
		CACC	98	241	553	402	143	68	363
2	CLZ_5	CACC	201	382	653	727	782	775	903
14	CLZ_6	CACT	169	1576	1400	727	987	933	909
		CAGA	119	388	129	217	102	115	119
65	CLZ_52	CAGA	146	737	728	643	511	354	332
		CAGA	157	927	820	422	943	533	893
		CAGA	214	118	94	79	129	229	163
		CAGC	247	508	1511	557	483	531	527
		CAGG	129	647	536	588	592	571	493
		CATA	172	534	482	447	494	863	625
3	CLZ_8	CATC	98	94	333	253	141	76	212
		CATC	135	350	483	606	403	299	464
		CATG	78	78	58	56	98	126	217
		CATG	197	406	401	421	474	427	318
64	CLZ_51	CATG	247	1740	1436	2195	3089	2713	4020
		CATT	420	194	114	155	122	259	214
		CATT	429	119	89	96	105	198	141
		CATT	432	127	101	106	104	229	157
		CCAC	404	28	12	23	37	51	93
		CCAG	87	58	29	28	115	100	229
4	CLZ_10	CCAG	104	211	309	353	154	153	262
		CCAT	119	122	38	91	35	113	179
		CCAT	133	57	45	66	59	95	100
		CCAT	296	16	34	7	8	80	56
		CCAT	440	56	76	86	104	83	97
		CCCC	123	474	860	910	628	277	698
		CCCG	243	163	654	354	120	146	129
		CCCG	277	218	282	257	310	660	337
		CCCG	283	298	261	421	250	779	323
		CCCG	454	84	69	115	90	140	102
		CCCT	119	107	76	104	146	176	132
		CCGC	88	32	231	134	82	843	226
		CCGC	93	197	52	18	743	462	367
		CCGC	118	2960	2515	1919	1789	1038	540
		CCGC	309	153	126	94	78	164	156
		CCGG	89	201	406	535	612	446	377
61	CLZ_48	CCGG	94	176	705	527	578	482	702

		CCGG	249	563	188	384	393	295	487
		CCGG	263	535	275	183	219	309	161
		CCGT	169	363	246	408	247	559	398
5	CLZ_12	CCGT	172	765	511	343	347	407	174
63	CLZ_50	CCGT	293	88	57	65	52	426	251
		CCGT	350	82	24	91	37	52	100
		CCTA	110	174	342	363	204	214	195
		CCTA	379	80	89	170	105	192	217
		CCTC	382	72	83	88	66	105	110
		CCTG	99	283	93	245	1081	319	379
		CCTG	130	1413	1995	1550	934	1004	1180
		CCTT	104	304	533	768	344	288	0
		CGAA	101	66	225	382	71	130	305
		CGAC	76	71	45	704	87	174	1047
		CGAC	148	1008	1239	1016	884	1043	999
		CGAC	480	556	498	421	605	1183	913
		CGAC	490	317	250	225	282	531	473
		CGAG	273	212	98	136	89	96	136
		CGAG	450	122	122	101	173	230	181
		CGAT	78	322	85	178	293	484	420
		CGAT	95	42	40	62	80	94	50
		CGAT	98	48	62	67	68	124	52
		CGAT	105	97	59	45	199	206	151
		CGAT	268	770	202	374	593	519	478
		CGAT	496	170	164	127	196	147	146
		CGCA	88	592	249	355	696	542	854
		CGCA	334	1071	1923	1725	1333	1445	1438
		CGCA	472	218	306	294	365	312	406
		CGCG	82	61	115	148	377	254	133
		CGCG	85	32	115	60	275	248	133
		CGCG	111	49	236	266	826	778	323
		CGCG	371	27	37	72	44	101	56
		CGCT	118	905	634	948	855	668	542
		CGCT	341	22	29	39	11	62	23
		CGGC	87	66	89	149	216	198	150
		CGGC	110	311	620	1099	292	124	687
		CGGG	85	259	928	777	314	252	437
		CGGG	102	35	35	175	93	365	99
		CGGG	109	34	28	63	65	112	96
		CGGG	135	100	203	120	91	434	537
		CGGG	402	116	116	170	205	226	178
		CGGG	490	59	69	116	116	142	100
		CGGT	142	207	147	171	201	301	322

6	CLZ_15	CGGT	217	174	116	130	91	87	83
		CGGT	476	46	30	29	41	60	53
		CGTC	342	71	87	121	79	393	92
		CGTG	124	346	240	174	115	144	168
		CGTG	234	346	131	129	105	71	119
		CGTG	306	796	1334	1296	1163	1164	1114
		CGTT	81	42	91	35	129	186	74
		CGTT	245	169	161	216	168	402	185
		CTAA	268	125	133	121	157	151	201
58	CLZ_43	CTAA	461	120	131	146	185	397	220
		CTAC	93	90	73	124	101	146	106
		CTAC	359	184	161	249	238	357	258
		CTAG	91	48	29	64	113	142	175
		CTAG	97	360	331	395	116	102	537
15	CLZ_16	CTAG	171	412	247	167	119	181	142
		CTAT	190	61	41	67	59	89	74
49	CLZ_17	CTCA	206	567	522	466	306	370	239
		CTCA	313	39	19	47	36	51	55
		CTCG	140	90	94	293	259	663	605
		CTCG	218	1262	450	734	340	124	208
		CTCG	331	59	28	84	49	88	104
		CTCG	490	352	257	320	376	616	504
		CTCG	498	258	152	234	315	597	488
		CTCT	137	503	422	462	762	965	828
		CTCT	142	1146	797	1258	1620	1881	1685
		CTGA	115	29	30	42	30	130	55
62	CLZ_49	CTGA	450	127	173	228	279	258	265
		CTGC	116	0	449	479	212	188	0
57	CLZ_18	CTGC	320	0	60	83	99	104	0
		CTGG	84	102	54	62	90	117	126
		CTGG	183	269	195	328	321	308	1166
		CTTA	86	49	24	69	48	73	52
		CTTA	132	58	45	58	60	97	58
		CTTA	378	297	350	416	443	747	450
		CTTA	494	31	24	39	24	56	44
		CTTA	499	10	29	45	42	69	52
		CTTC	77	26	30	49	58	64	45
		CTTG	83	792	397	700	601	967	1173
		CTTG	176	119	75	200	187	192	229
		GAAC	78	35	17	117	36	36	51
		GAAG	93	122	348	230	116	116	183
		GAAG	148	552	569	635	454	343	560
		GAAG	196	363	237	448	447	223	350

		GAAG	223	44	31	51	63	71	101
		GAAG	226	44	31	51	62	71	81
		GAAG	231	18	15	30	31	71	85
		GACG	79	26	20	38	47	57	62
		GACG	97	597	409	195	127	214	160
		GACG	423	187	294	260	280	377	377
		GACT	155	117	111	137	201	241	147
		GAGG	103	136	175	399	79	90	139
		GAGG	248	227	82	85	120	112	117
		GAGT	367	302	382	345	369	355	326
		GATA	345	15	33	31	50	94	30
		GATC	95	81	170	177	112	67	130
		GATC	356	34	35	67	48	108	42
		GATG	300	375	310	202	280	270	293
		GATT	91	50	18	32	41	40	55
		GCAA	90	211	210	261	303	206	194
		GCAA	269	222	90	150	140	218	237
		GCAC	92	63	82	119	59	416	266
		GCAC	186	282	238	186	308	203	156
		GCAT	121	229	260	229	149	166	222
		GCAT	439	19	25	28	34	57	35
		GCCA	112	189	312	216	134	102	213
		GCCA	240	49	47	22	27	119	68
		GCCC	79	60	42	40	62	89	101
		GCCC	121	62	42	39	57	96	212
		GCCC	294	695	144	403	428	422	469
67	CLZ_56	GCCC	324	202	648	578	521	512	802
		GCCG	139	57	36	128	115	146	87
		GCCG	144	78	39	71	52	101	139
		GCCT	84	122	68	102	166	150	165
		GCCT	118	403	671	853	366	337	489
		GCCT	126	561	294	305	328	188	246
		GCGA	180	235	1349	636	733	1018	1159
		GCGA	293	1031	312	375	643	332	335
68	CLZ_57	GCGC	325	35	61	60	75	104	95
		GCGG	77	65	79	91	73	193	78
		GCGG	127	51	50	52	107	161	130
		GCGG	254	413	167	190	231	214	251
		GCGG	269	842	133	372	326	480	586
		GCGG	471	93	130	112	129	149	147
		GCGT	140	117	55	78	115	189	159
		GCGT	168	701	465	504	599	429	405
		GCGT	309	498	282	77	186	71	139

		GCTA	109	388	639	619	320	267	550
		GCTA	132	990	829	1198	735	669	968
		GCTA	223	898	532	586	525	812	522
16	CLZ_22	GCTA	292	444	169	168	171	182	154
		GCTC	174	100	26	34	57	109	114
		GCTC	202	785	866	512	626	949	593
		GCTC	326	752	666	793	862	890	1479
		GCTG	78	103	116	427	446	587	312
		GCTG	120	1694	2136	2033	1141	1119	1652
		GCTG	172	31	31	116	154	114	74
		GCTT	233	43	20	62	23	51	63
		GGAA	434	49	114	93	142	230	125
		GGAC	231	683	585	478	510	254	236
		GGAC	472	62	50	62	68	112	120
		GGAG	221	423	239	203	217	250	248
		GGAG	372	836	772	775	1052	913	641
		GGAT	223	1048	1430	1425	1632	944	1461
		GGCA	305	155	124	206	194	280	164
7	CLZ_24	GGCA	393	303	544	393	608	725	842
		GGCC	113	334	371	479	204	175	240
		GGCC	134	838	720	633	537	668	608
		GGCC	324	114	115	211	157	238	301
		GGCC	418	40	12	32	28	26	52
		GGCG	113	235	158	129	129	130	101
		GGCG	136	97	61	76	59	125	145
		GGCG	315	292	238	445	464	495	366
50	CLZ_26	GGCT	129	491	544	423	199	169	321
69	CLZ_60	GGCT	169	467	563	335	704	1233	1055
		GGCT	176	127	173	164	410	407	230
		GGGA	172	91	97	67	144	112	183
		GGGA	377	307	157	252	269	263	255
		GGGC	214	59	62	85	66	252	255
		GGGC	286	27	23	34	29	60	71
		GGGG	81	670	1443	1269	1095	2164	1645
		GGGT	91	63	68	104	267	143	91
		GGTA	128	265	198	142	124	153	146
		GGTA	184	1209	969	875	1109	836	941
51	CLZ_28	GGTA	257	1016	872	549	492	539	422
		GGTG	139	992	884	936	801	733	811
		GGTT	100	12	17	35	32	41	94
		GTAA	257	86	36	105	119	75	98
		GTAC	107	815	975	1034	821	751	1057
		GTAG	244	260	237	294	349	736	282

		GTAG	459	113	137	168	239	351	199
		GTAG	459	113	137	168	239	351	199
		GTAG	471	75	68	76	103	172	99
		GTCC	87	448	256	218	325	193	176
		GTCC	124	111	443	155	139	104	160
		GTCC	187	1253	1031	1066	1018	891	778
		GTCC	413	28	29	42	35	61	42
		GTCG	176	55	58	79	190	130	126
		GTCG	228	3085	2559	3211	3000	3470	3051
		GTCT	84	19	28	30	43	128	35
		GTGC	87	58	106	159	316	867	410
		GTGG	125	1407	1734	1004	1276	1047	1475
		GTGG	147	821	314	343	174	188	188
		GTGG	458	45	22	41	35	26	33
		GTTC	491	90	236	206	175	240	176
		GTTG	93	156	129	90	93	150	88
		GTTG	114	20	37	44	58	75	78
		GTTG	378	66	35	74	59	80	73
		GTTT	260	49	24	33	42	56	49
		GTTT	336	37	42	40	36	139	126
		GTTT	339	31	37	40	34	156	108
		GTTT	495	36	23	34	54	58	50
		TAAA	84	27	25	46	37	60	37
		TAAC	114	38	32	50	48	65	41
		TAAC	222	411	367	454	384	216	229
		TAAC	450	678	538	407	452	753	669
		TAAG	386	210	334	126	421	702	301
		TACA	119	42	49	73	98	111	103
		TACA	129	282	242	227	197	206	180
		TACA	200	801	493	438	442	477	324
		TACC	99	132	141	88	51	18	102
		TACC	129	185	160	327	486	457	247
		TACC	169	122	72	83	103	179	255
		TACC	344	88	71	89	79	104	183
17	CLZ_32	TACG	274	181	206	160	187	255	578
		TACT	151	94	34	53	44	97	132
8	CLZ_33	TACT	188	184	278	1200	581	339	347
		TACT	386	36	50	70	56	104	88
		TAGA	125	41	88	152	95	195	106
		TAGA	134	286	263	214	194	146	152
		TAGA	242	32	9	26	37	142	51
		TAGC	186	1357	1306	1263	1125	959	889
		TAGC	411	56	68	76	76	142	123

		TAGC	415	50	60	40	66	127	87
		TAGC	464	183	184	166	133	129	106
		TAGG	250	461	166	238	189	306	257
		TAGT	81	213	160	178	286	473	369
		TAGT	97	271	144	246	309	537	299
		TATA	98	115	183	488	127	99	230
		TATA	382	37	36	49	44	113	39
72	CLZ_65	TATC	159	434	327	334	404	701	2760
		TATC	262	119	154	204	168	826	154
71	CLZ_62	TATG	290	135	103	59	121	37	52
		TATG	446	201	229	389	325	462	328
9	CLZ_34	TATT	89	156	623	509	129	186	314
		TATT	112	50	38	182	101	122	50
		TATT	119	43	16	25	52	40	43
		TATT	230	403	42	24	31	35	103
		TATT	272	59	43	59	57	131	88
		TATT	354	44	36	63	42	147	99
		TCAA	447	44	38	39	26	85	49
		TCAC	134	836	1637	842	57	1228	1047
		TCAC	212	777	567	742	688	573	552
		TCAC	289	1707	1138	1116	842	943	1123
		TCAG	84	56	68	205	125	148	108
		TCAT	88	88	145	178	409	401	430
18	CLZ_36	TCAT	349	2478	380	1155	1425	903	1832
70	CLZ_64	TCAT	391	314	216	421	391	554	699
		TCAT	473	45	22	38	39	53	47
		TCCA	106	150	71	193	91	179	385
		TCCA	222	400	303	362	613	787	616
		TCCA	435	68	78	56	57	241	71
		TCCA	439	54	78	56	61	174	71
10	CLZ_37	TCCC	97	381	1687	1532	720	673	1083
		TCCC	148	1050	865	963	700	639	685
		TCCG	120	0	832	774	566	649	653
		TCCG	185	0	311	292	223	206	259
		TCCT	98	577	621	882	925	1258	1741
		TCCT	144	492	551	427	580	313	410
		TCCT	166	740	488	588	605	421	473
		TCCT	275	72	20	77	52	108	133
		TCGA	255	533	263	431	473	614	575
		TCGA	370	167	148	178	194	215	229
		TCGC	196	229	155	214	97	412	311
		TCGC	328	465	545	856	482	674	773
		TCGT	326	32	32	95	33	85	34

		TCTA	80	49	65	184	75	563	231
		TCTA	217	39	50	160	325	212	84
		TCTC	143	341	256	203	262	229	141
		TCTT	155	93	80	96	91	252	110
		TGAA	240	542	390	530	667	552	540
		TGAC	193	1029	566	798	752	902	1048
19	CLZ_42	TGAC	328	194	216	199	314	475	303
		TGAT	97	45	44	23	72	158	85
		TGAT	138	608	468	542	442	467	498
11	CLZ_38	TGCA	109	339	554	561	473	736	395
		TGCA	185	137	83	67	160	382	346
		TGCC	163	271	347	93	330	958	407
		TGCC	185	1164	1680	573	1081	1145	992
		TGCC	343	604	628	832	675	889	1068
		TGCG	77	188	156	495	125	366	403
		TGCG	111	36	50	76	225	167	155
		TGGA	93	173	157	202	253	545	240
		TGGA	108	1941	294	2077	1692	1853	2640
		TGGA	154	823	1504	1481	1370	1122	673
		TGGA	277	50	23	54	56	103	93
		TGGA	308	31	32	52	51	149	84
		TGGC	105	634	538	630	818	1092	669
		TGGC	113	377	259	371	510	524	415
		TGGC	160	156	213	282	223	460	320
		TGGC	266	468	451	365	280	207	270
		TGGC	276	73	81	59	81	251	274
		TGGC	494	98	43	27	58	88	122
		TGGG	93	33	65	48	55	228	583
		TGGG	271	241	591	580	426	642	607
		TGGT	103	76	25	97	93	132	236
		TGGT	114	339	537	421	221	204	231
		TGGT	122	119	145	180	135	341	182
		TGGT	158	465	286	403	324	267	348
		TGGT	330	666	673	726	770	701	753
		TGTA	121	1021	1596	1727	1052	696	1206
		TGTA	169	1562	681	624	801	880	753
		TGTC	84	160	250	216	410	510	399
		TGTC	109	711	704	686	276	149	466
		TGTG	315	71	56	83	35	125	73
		TGTG	393	430	313	425	528	419	664
		TGTG	450	573	554	698	819	1166	654
		TGTT	114	335	752	657	875	794	838
		TGTT	119	703	1167	993	1666	1824	1251

		TGTT	453	138	226	333	307	324	287
		TTAA	88	149	109	181	377	239	326
		TTAA	194	369	115	230	262	391	313
		TTAA	312	335	177	159	199	136	167
		TTAC	174	287	294	137	192	196	180
		TTAG	104	52	51	54	44	112	65
		TTAT	106	41	22	50	232	53	44
		TTAT	338	486	777	852	875	816	884
		TTCC	96	97	140	133	130	370	135
		TTCC	104	51	31	109	67	94	78
		TTGA	117	20	28	34	38	63	60
		TTGC	119	57	52	67	73	117	75
		TTGC	299	151	114	68	60	65	59
		TTGG	209	704	1160	894	921	857	1215
		TTGG	466	60	47	46	71	103	68
12	CLZ_40	TTGT	266	200	52	75	82	67	115
		TTGT	302	38	33	72	48	69	79
		TTGT	483	53	87	120	110	140	60
		TTTA	249	174	32	103	46	55	85
		TTTC	85	31	44	34	89	369	100
		TTTC	107	50	37	20	65	91	68
		TTTC	118	633	721	715	303	257	483
		TTTC	153	188	168	113	141	142	270
		TTTC	171	663	642	709	704	801	589
		TTTC	226	26	31	22	63	63	86
		TTTC	277	566	324	375	327	381	278

TABLE 2

Seq ID	Clone ID	Digital Address (Msp1)	Database Match (Accession #)	% Homology	Nucleotide Homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
1	CLZ_3	AGTA 106	Mus musculus serine protease HTRA mRNA, complete cds (AF172994.1) and Mus musculus insulin-like growth factor binding protein 5 protease (AF179369.1)	100% 100%	1 – 46 1 – 46	1965 – 2010 1960 – 2005
2	CLZ_5	CACC 201	Mouse mRNA for apolipoprotein D (X82648)	99%	1 – 149	433 – 581
3	CLZ_8	CATC 98	(EST) UI-M-AN1-afi-g-11-0-UI.s1 NIH_BMAP_MBG_N Mus musculus cDNA clone/UI-M-AN1-afi-g-11-0-UI 3', mRNA sequence (AI846711.1)	95%	1 – 48	10 – 57
4	CLZ_10	CCAG 104	(EST) mf92h11.x1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE 421797 3', mRNA sequence [Mus musculus] (AI429767)	98%	1 – 55	99 – 153
5	CLZ_12	CCGT 172	Mus musculus importin alpha Q1 mRNA, complete cds (AF020771)	100%	8 – 119	2 – 113
6	CLZ_15	CGGT 217	Mus musculus dystroglycan (Dag1) mRNA (U43512)	98%	1 – 160	3012 – 3170
7	CLZ_24	GGCA 393	(EST) UI-R-C1-ld-g-08-0-UI-.s1 UI-R-C1 Rattus norvegicus cDNA clone UI-R-C1-ld-g-08-0-UI 3', mRNA sequence [Rattus norvegicus] (AI502824)	90%	1 – 340	20 – 356

TABLE 2 (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Database Match (Accession #)	% Homology	Nucleotide Homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
8	CLZ_33	TACT 188	(EST) Mus musculus C57BL/6J 10-day embryo Mus musculus cDNA clone 2610203G07, mRNA sequence (AV117493.1)	95%	1 – 131	74 – 204
9	CLZ_34	TATT 89	Rattus norvegicus Sprague-Dawley N-methyl-D-aspartate receptor NMDAR1-2a subunit (NMDAR1) mRNA, complete cds (U08262)	100%	1 – 32	4039 – 4070
10	CLZ_37	TCCC 97	(EST) UI-M-AH1-agt-h-06-0-UI.s1 NIH-BMAP-MCE-N Mus musculus cDNA clone UI-M-AH1-agt-h-06-0-UI 3', mRNA sequence (AI849537.1)	100%	1 – 45	13 – 57
11	CLZ_38	TGCA 109	Mus musculus oligodendrocyte-specific protein mRNA, complete cds (U19582)/(AR009501) Sequence 1 from patent U.S. 5756300	100%	1 – 48	1745 – 1792
12	CLZ_40	TTGT 266	(EST) vx01g05.x1 Soares 2NbMT Mus musculus cDNA clone IM mRNA (AI549943.1)	99%	1 – 205	4 – 208
14	CLZ_6	CACT 169	Mus musculus LIM-kinase1 (Limk1) gene, complete cds; Wbscr1 (Wbscr1) gene, alternative splice products, complete cds; and replication factor C, 40kDa subunit (Rfc2) gene, complete cds (AF139987.1)	86%	3 – 118	7957 – 8072
15	CLZ_16	CTAG 171	Mus musculus arm-repeat protein NPRAP/neurojungin (Nprap) mRNA (U90331.1)	99%	1 – 119	2845 – 2963
16	CLZ_22	GCTA 292	(EST) vk75e05.s1 Knowles Solter mouse 2 cell Mus musculus 960512 5' (AA549416)	99%	5 – 211	209 – 415

TABLE 2 (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Database Match (Accession #)	% Homology	Nucleotide Homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
17	CLZ_32	TACG 274	Mus musculus high mobility group protein I-C gene, exon 5 (L41622) and Mus musculus early blastocyst cDNA, clone 01B00056NM07 (C89064)	95% 97%	89 – 152 1 – 215	1 – 65 245 – 459
18	CLZ_36	TCAT 349	Homology to rat mRNA for mitochondrial enoyl-CoA hydratase (EC4.2.1.17) (X15958)	94%	1 – 298	397 – 694
19	CLZ_42	TGAC 328	(EST) UI-M-AN1-afc-b-05-0-UI.s1 Mus musculus cDNA clone (AI843761.1)	98%	1 – 271	20 – 290
57	CLZ_18	CTGC 320	(EST) mj75b02.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 481899 5' (AA059879)	97%	1 – 271	91 – 361
58	CLZ_43	CTAA 461	(EST) ud25c08.r1 Soares_thymus_2NbMT Mus musculus cDNA clone (AI158519.1)	99%	1 – 396	1 – 396
59	CLZ_44	ACGG 352	(EST) uj37f10.x1 Sugano mouse kidney mkia Mus musculus cDNA clone IMAGE:1922155 3' similar to TR:Q14120 Q14120 DBP-5 NUCLEAR PROTEIN, mRNA sequence [Mus musculus] (AI315677)	98%	1 – 298	84 – 381
60	CLZ_47	AATA 136	Homology to Homo sapiens Bcl-2 associated transcription factor short form mRNA , complete cds. (AF249273.1)	96%	1 – 81	1279 – 1359
61	CLZ_48	CCGG 94	(EST) UI-M-BH3-arb-e-09-0-UI.s1 NIH_BMAP_M_S4 Mus musculus cDNA clone (AW457685.1)	97%	1 – 42	13 – 54
62	CLZ_49	CTGA 450	Mus musculus autoantigen La (SS-B) mRNA, complete cds (L00993.1)	100%	1 – 397	318 – 714

TABLE 2 (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Database Match (Accession #)	% Homology	Nucleotide Homology	
					DST nucleotide range (bp#)	Database nucleotide range (bp#)
63	CLZ_50	CCGT 293	(EST) uj28f11.xi Sugano mouse kidney mkia Mus musculus cDNA clone IMAGE:1921293 3' (AI315041.1)	97%	7 – 237	96 – 326
64	CLZ_51	CATG 247	(EST) vm08c06.r1 Knowles Solter mouse blastocyst B1 Mus musculus cDNA clone IMAGE:989578 (AA571556.1)	100%	1 – 190	5 – 194
65	CLZ_52	CAGA 146	NOVEL	N/A	N/A	N/A
67	CLZ_56	GCCC 324	Homology to R. rattus (Sprague-Dawley) mRNA for brain myosin II isoform (810bp) (Z32518.1)	93%	1 – 265	46 – 310
68	CLZ_57	GCGC 325	(EST) G0109H07-3 Mouse E7.5 Embryonic Portion cDNA Library Mus musculus cDNA clone G0109H07 (AW536880.1)	99%	1 – 268	305 – 572
69	CLZ_60	GGCT 169	Homology to (EST) UI-R-C2-mv-g-10-0-UI.s1 UI-R-C2 Rattus norvegicus cDNA clone UI-R-C2-mv-g-10-0-UI (AI070642.1)	95%	3 – 103	29 – 125
71	CLZ_62	TATG 290	(EST) vp20e08.r1 Soares_mammary_gland_NbMMG Mus musculus cDNA clone IMAGE:1069190 5' similar to SW:YBF5_YEAST P34220 HYPOTHETICAL 47.4 KD PROTEIN IN PTC3-SEC17 INTERGENIC REGION (AA792913.1)	99%	1 – 219	10 – 228

TABLE 2 (continued)

Seq ID	Clone ID	Digital Address (Msp1)	Database Match (Accession #)	% Homology	Nucleotide Homology	
					DST nucleotide range (bp#)	DST nucleotide range (bp#)
70	CLZ_64	TCAT 391	Homology to rat mRNA for mitochondrial enoyl-CoA hydratase (EC 4.2.1.17) (X15958) and	92%	1 – 338	397 – 734
			(EST) mx28c10.r1 Soares mouse NML Mus musculus cDNA clone 681522 5'similar to SW:ECHM_RAT P14604 ENOYL-COA HYDRATASE, MITOCHONDRIAL PRECURSOR (AA237635)	96%	1 – 334	130 – 463
72	CLZ_65	TATC 159	Mus musculus Purkinje cell protein 4 (Pcp4), mRNA (NM_008791.1)	99%	2 – 102	374 – 474

EST = Expressed Sequence Tag, N/A = Not Applicable

TABLE 3

Clone ID	Digital Address (Msp1)	Database Match (Accession #)	Extended Primer	Seq ID
CLZ_3	AGTA 106	Mus musculus serine protease HTRA mRNA, complete cds (AF172994.1) and Mus musculus insulin-like growth factor binding protein 5 protease (AF179369.1)	GAT CGA ATC CGG AGT ACA GTG ACT TTG AGT	28
CLZ_5	CACC 201	Mouse mRNA for apolipoprotein D (X82648)	GAT CGA ATC CGG CAC CCT ACT GGA TCC TGG	29
CLZ_8	CATC 98	(EST) UI-M-AN1-afi-g-11-0-UI.s1 NIH_BMAP_MBG_N Mus musculus cDNA clone/UI-M-AN1-afi-g-11-0-UI 3', mRNA sequence (AI846711.1)	GAT CGA ATC CGG CAT CCA GCT GGA TGT CAG	30
CLZ_10	CCAG 104	(EST) mf92h11.x1 Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA clone IMAGE 421797 3', mRNA sequence [Mus musculus] (AI429767)	GAT CGA ATC CGG CCA GAG TCT GAT TAG GGC	31
CLZ_12	CCGT 172	Mus musculus importin alpha Q1 mRNA, complete cds (AF020771)	GAT CGA ATC CGG CCG TGG CGG ACA ACG AGA	32
CLZ_15	CGGT 217	Mus musculus dystroglycan (Dag1) mRNA (U43512)	GAT CGA ATC CGG CGG TGG CCA TCA GAC TCT	33
CLZ_24	GGCA 393	(EST) UI-R-C1-ld-g-08-0-UI-.s1 UI-R-C1 Rattus norvegicus cDNA clone UI-R-C1-ld-g-08-0-UI 3', mRNA sequence [Rattus norvegicus] (AI502824)	GAT CGA ATC CGG GGC AAG GAG CAC CAG AAG	34
CLZ_33	TACT 188	(EST) Mus musculus C57BL/6J 10-day embryo Mus musculus cDNA clone 2610203G07, mRNA sequence (AV117493.1)	GAT CGA ATC CGG TAC TCC GCT CTG ATC ATC	35

TABLE 3 (Continued)

Clone ID	Digital Address (Msp1)	Database Match (Accession #)	Extended Primer	
CLZ_34	TATT 89	Rattus norvegicus Sprague-Dawley N-methyl-D-aspartate receptor NMDAR1-2a subunit (NMDAR1) mRNA, complete cds (U08262)	GAT CGA ATC CGG TAT TCA GTG GTG ATG CCT	36
CLZ_37	TCCC 97	(EST) UI-M-AH1-agt-h-06-0-UI.s1 NIH-BMAP-MCE-N Mus musculus cDNA clone UI-M-AH1-agt-h-06-0-UI 3', mRNA sequence (AI849537.1)	GAT CGA ATC CGG TCC CTG CCG CTC AAT AAA	37
CLZ_38	TGCA 109	Mus musculus oligodendrocyte-specific protein mRNA, complete cds (U19582)/(AR009501) Sequence 1 from patent U.S. 5756300	GAT CGA ATC CGG TGC ATT TGT TCA GGT AAA	38
CLZ_40	TTGT 266	(EST) vx01g05.x1 Soares 2NbMT Mus musculus cDNA clone IM mRNA (AI549943.1)	GAT CGA ATC CGG TTG TGG TTC AGT GGC AAG	39
CLZ_6	CACT 169	Mus musculus LIM-kinase1 (Limk1) gene, complete cds; Wbscr1 (Wbscr1) gene, alternative splice products, complete cds; and replication factor C, 40kDa subunit (Rfc2) gene, complete cds (AF139987.1)	GAT CGA ATC CGG CAC TTG GGA GGC AGA GAC	40
CLZ_16	CTAG 171	Mus musculus arm-repeat protein NPRAP/neurojungin (Nprap) mRNA (U90331.1)	GAT CGA ATC CGG CTA GCA GCA GAA ACG TCT	41
CLZ_22	GCTA 292	(EST) vk75e05.s1 Knowles Solter mouse 2 cell Mus musculus 960512 5' (AA549416)	GAT CGA ATC CGG GCT AGA ACG CCA GCC AGA	42

TABLE 3 (Continued)

Clone ID	Digital Address (Msp1)	Database Match (Accession #)	Extended Primer	
CLZ_32	TACG 274	Mus musculus high mobility group protein I-C gene, exon 5 (L41622) and Mus musculus early blastocyst cDNA, clone 01B00056NM07 (C89064)	GAT CGA ATC CGG TAC GAT GCT GTG ACA ATT	43
CLZ_36	TCAT 349	Homology to rat mRNA for mitochondrial enoyl-CoA hydratase (EC4.2.1.17) (X15958)	GAT CGA ATC CGG TCA TCG CAG CTG TCA ATG	44
CLZ_42	TGAC 328	(EST) UI-M-AN1-afc-b-05-0-UI.s1 Mus musculus cDNA clone (AI843761.1)	GAT CGA ATC CGG TGA CAG ACA GAA GAG GAT	45
CLZ_18	CTGC 320	(EST) mj75b02.r1 Soares mouse p3NMF19.5 Mus musculus cDNA clone 481899 5' (AA059879)	GAT CGA ATC CGG CTG CAG GTG AGG GCT GGT	73
CLZ_43	CTAA 461	(EST) ud25c08.r1 Soares_thymus_2NbMT Mus musculus cDNA clone (AI158519.1)	GAT CGA ATC CGG CTA ATA TTG ATA ATC TTT	74
CLZ_44	ACGG 352	(EST) uj37f10.x1 Sugano mouse kidney mkia Mus musculus cDNA clone IMAGE:1922155 3' similar to TR:Q14120 Q14120 DBP-5 NUCLEAR PROTEIN, mRNA sequence [Mus musculus] (AI315677)	GAT CGA ATC CGG ACG GTG TAC CCC GAG GAT	75
CLZ_47	AATA 136	Homology to Homo sapiens Bcl-2 associated transcription factor short form mRNA , complete cds. (AF249273.1)	GAT CGA ATC CGG AAT ACT GAG GAG GAA GGA	76
CLZ_48	CCGG 94	(EST) UI-M-BH3-arb-e-09-0-UI.s1 NIH_BMAP_M_S4 Mus musculus cDNA clone (AW457685.1)	GAT CGA ATC CGG CCG GCA TGA AAT AAA ACA	77

TABLE 3 (Continued)

Clone ID	Digital Address (Msp1)	Database Match (Accession #)	Extended Primer	
CLZ_49	CTGA 450	Mus musculus autoantigen La (SS-B) mRNA, complete cds (L00993.1)	GAT CGA ATC CGG CTG ACA ACA GAC TTT AAT	78
CLZ_50	CCGT 293	(EST) uj28f11.xi Sugano mouse kidney mkia Mus musculus cDNA clone IMAGE:1921293 3' (AI315041.1)	GAT CGA ATC CGG CCG TGG TGG CGC ACA CCA	79
CLZ_51	CATG 247	(EST) vm08c06.r1 Knowles Solter mouse blastocyst B1 Mus musculus cDNA clone IMAGE:989578 (AA571556.1)	GAT CGA ATC CGG CAT GGG TGG TCT TCA TCC	80
CLZ_52	CAGA 146	NOVEL	GAT CGA ATC CGG CAG ACC TAG CTC AGC TTG	81
CLZ_56	GCCC 324	Homology to R. rattus (Sprague-Dawley) mRNA for brain myosin II isoform (810bp) (Z32518.1)	GAT CGA ATC CGG GCC CCA TCA ATT TCA CCA	82
CLZ_57	GCGC 325	(EST) G0109H07-3 Mouse E7.5 Embryonic Portion cDNA Library Mus musculus cDNA clone G0109H07 (AW536880.1)	GAT CGA ATC CGG GCG CCA TCA ATT TCA CCA	83
CLZ_60	GGCT 169	Homology to (EST) UI-R-C2-mv-g-10-0-UI.s1 UI-R-C2 Rattus norvegicus cDNA clone UI-R-C2-mv-g-10-0-UI (AI070642.1)	GAT CGA ATC CGG GGC TCA AAG ACA AGG GTT	84
CLZ_62	TATG 290	(EST) vp20e08.r1 Soares_mammary_gland_NbMMG Mus musculus cDNA clone IMAGE:1069190 5' similar to SW:YBF5_YEAST P34220 HYPOTHETICAL 47.4 KD PROTEIN IN PTC3-SEC17 INTERGENIC REGION (AA792913.1)	GAT CGA ATC CGG TAT GTG GGT AGA GTG GTC	85

TABLE 3 (Continued)

Clone ID	Digital Address (Msp1)	Database Match (Accession #)	Extended Primer	
CLZ_64	TCAT 391	Homology to rat mRNA for mitochondrial enoyl-CoA hydratase (EC 4.2.1.17) (X15958) and (EST) mx28c10.r1 Soares mouse NML Mus musculus cDNA clone 681522 5'similar to SW:ECHM_RAT P14604 ENOYL-COA HYDRATASE, MITOCHONDRIAL PRECURSOR (AA237635)	GAT CGA ATC CGG TCA TCG CAG CTG TCA ATG	86
CLZ_65	TATC 159	Mus musculus Purkinje cell protein 4 (Pcp4), mRNA (NM_008791.1)	GAT CGA ATC CGG TAT CCA CAG TAA AAT TGT	87

EST = Expressed Sequence Tag

TABLE 4: VERIFIED CANDIDATE MATCHES

Seq ID	Clone ID	Digital Address (MspI)	Gene Identity (Accession #)	Extended Primer
49	CLZ_17	CTCA 206	Consensus sequence based on Computer Assembled ESTs: Soares mouse p3NMF19.5 Mus musculus cDNA clone IMAGE:350746 3', mRNA sequence (AI415388) UI-M-AM0-ado-e-04-0-UI.s1 NIH_BMAP_MAM Mus musculus cDNA clone UI-M-AM0-ado-e-04-0-UI 3', mRNA sequence (AI841003) Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:356159 3', mRNA sequence (AI413353) Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:426077 3', mRNA sequence (AI425991)	GAT CGA ATC CGG CTC AGC ACT TGG CAG CTG (SEQ ID NO:46)
50	CLZ_26	GGCT 129	Mus musculus metalloprotease-disintegrin MDC15 mRNA, complete cds (AF006196)	GAT CGA ATC CGG GGC TGG AGT AGG TGG CGG (SEQ ID NO:47)

TABLE 4 (Continued)

51	CLZ_28	GGTA 257	Consensus sequence based on Computer Assembled ESTs: Mus musculus fertilized egg cDNA 3'-end sequence, clone J0229E09 3', mRNA sequence (C86593) Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone IMAGE:553802 3', mRNA sequence (AI428410) Stratagene mouse skin (#937313) Mus musculus cDNA clone IMAGE:1227449 3', mRNA sequence (AI561814)	GAT CGA ATC CGG GGT AGG GAC ACC CCT GTA (SEQ ID NO:48)
52	CLZ_58	GATC 258	Mus musculus OG-12a homeodomain protein (OG-12) mRNA (U66918)	GAT CGA ATC CGG GAT CCC ACG AGG GCC ACC (SEQ ID NO:49)

TABLE 5A

Seq ID	Clone ID	Digital Address (Msp1)	Gene Match (Accession #)	Relative DST Amount						Validation Method
				Control	45 Minutes	7 Hour	5 Day	12 Day	14 Day	
12	CLZ_40	TTGT 266	(EST) VX01g05X1 Soares 2NbMT Mus musculus cDNA clone IM mRNA (AI549943.1)	100	18.2	29.3	24.3	43.4	41.4	N

TABLE 5B

Seq ID	Clone ID	Digital Address (Msp1)	Gene Match (Accession #)	% Homology	Nucleotide homology	
					Extended Seq. nucleotide range (bp#)	Database nucleotide range (bp#)
13	CLZ_40 Extended Sequence	TTGT 266	Soares 2NbMT Mus musculus cDNA clone IMAGE: mRNA sequence [Mus musculus] (AI509550)	98%	180 – 682	1 – 503

EXAMPLE 4
Characterization of CLZ 34

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses described below.

The TOGA data shown in Figure 18 was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-A-T-T; SEQ ID NO: 27) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

The results of TOGA analysis using a 5' PCR primer with parsing bases C-G-A-C-G-G-T-A-T-C-G-G-T-A-T-T (SEQ ID NO: 27) are shown in Figure 18, which shows PCR products produced from mRNA isolated from the striatum/nucleus accumbens of mice treated with clozapine for various lengths of time as described in Example 1. In Fig. 18, the vertical index line indicates a PCR product of about 89 b.p. that is present in control cells, and whose expression in the striatum/nucleus accumbens of mice treated with clozapine is differntially regulated with acute treatment versus chronic treatment. CLZ_34 is upregulated with clozapine treatment at 45 minutes and 7 hours, but decreases to control level by day 5 and remains at about control level for as long as 12 days, showing a slight increase at day 14. *In situ* analysis performed using CLZ_34 as a probe revealed that CLZ_34 is expressed ubiquitously throughout the brain (data not shown).

CLZ_34 corresponds with GenBank sequence UO8262, which is identified as a rat N-methyl-D-aspartate receptor/NMDAR1-2a subunit (NMDAR1). The NMDAR1 receptor is a glutamate receptor involved in the processes underlying learning and memory. In addition, numerous studies show that blockade of glutamate actions by noncompetitive (e.g. MK801 and dextromethorphan) and competitive (e.g.

LY274614) NMDA receptor antagonists blocks or reduces the development of morphine tolerance following long term opiate administration (Trujillo et al., *Science*, 251, 85-87, (1991); Elliott et al., *Pain*, 56, 69-75 (1994); Wiesenfeld-Hallin, Z., *Neuropsychopharm.*, 13, 347-56 (1995)). The early change in the level of expression of CLZ_34 which has high homology with an NMDA receptor is interesting in view of the ability of NMDA antagonists to block the development of tolerance to opioids.

EXAMPLE 5

Figure 19 shows the consensus sequence from the computer generated assembly of the following 4 sequences AI415388: Soares mouse p3NMF19.5 Mus musculus cDNA clone IMAGE:350746 3', mRNA sequence; AI841003: UI-M-AM0-ado-e-04-0-UI.s1 NIH_BMAP_MAM Mus musculus cDNA clone UI-M-AM0-ado-e-04-0-UI 3', mRNA sequence; AI413353: Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:356159 3', mRNA sequence; AI425991: Soares mouse embryo NbME13.5 14.5 Mus musculus cDNA IMAGE:426077 3', mRNA sequence. (SEQ ID NO: 53)

Figure 20 shows the sequence of the EST AF006196: Mus musculus metalloprotease-disintegrin MDC15 mRNA, complete cds. (SEQ ID NO: 54)

Figure 21 shows the consensus sequence from the computer generated assembly of the following 3 sequences: C86593: Mus musculus fertilized egg cDNA 3'-end sequence, clone J0229E09 3', mRNA sequence; AI428410: Life Tech mouse embryo 13 5dpc 10666014 Mus musculus cDNA clone IMAGE:553802 3', mRNA sequence; AI561814: Stratagene mouse skin (#937313) Mus musculus cDNA clone IMAGE:1227449 3', mRNA sequence. (SEQ ID NO: 55).

EXAMPLE 6
Characterization of CLZ 44

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-C-G-G; SEQ ID NO:96) paired with

the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_44 is slightly up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_44 is an EST isolated from mouse kidney. In further characterization of CLZ_44, northern blot analyses were performed to determine the pattern of expression in the striatum/nucleus accumbens after 2 weeks of treatment of control mice, clozapine-treated mice, haloperidol-treated mice, and ketanserin-treated mice (Figure 22). Ketanserin is a $5HT_{2A/2C}$ - selective antagonist, and is used to determine serotonorgic involvement in these drug effects.

Briefly, an agarose gel containing 2μg of poly A enriched mRNA as well as size standards was electrophoresed on a 1.5% agarose gel containing formaldehyde, transferred to a biotrans membrane, and prehybridized for 30 minutes in Expresshyb (Clonetech). A CLZ_44 insert (25-100 ng) was labeled with [α-^{32}P]-d CTP by oligonucleotide labeling to specific activities of approximately $5x10^8$ cpm/μg and added to the prehybridization solution and incubated 1 hour. Filters were washed to high stringency (0.2 X SSC) (1 X SSC: 0.015 M NaCl and 0.0015 M Na citrate) at 68°C then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) for up to 1 week. Figure 22 is a graphical representation of the described northern blot analyses. As shown, after 2 weeks of treatment, CLZ_44 was up-regulated with haloperidol and ketanserin, but not clozapine. This suggests that both dopamines D2 and $5HT_{2A/2C}$ receptors are involved in CLZ_44 expression regulation. The lack of effect of clozapine may indicate that antagonism at other receptors (i.e. $5HT_3$, D4, D1) may override the effects of D2, $5HT_2$ receptors.

EXAMPLE 7
Characterization of CLZ 38

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine

treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-G-C-A; SEQ ID NO: 97) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

Tables 2 and 3 show that CLZ_38 is an oligodendrocyte-specific protein mRNA. In further characterization of CLZ_38, northern blot analyses were performed to determine the pattern of expression in the striatum/nucleus accumbens of control miceand mice treated with clozapine for 45 minutes, 7 hours, 5 days, and 2 weeks (Figure 23).

Briefly, an agarose gel containing 2μg of poly A enriched mRNA as well as size standards was electrophoresed on a 1.5% agarose gel containing formaldehyde, transferred to a biotrans membrane, and prehybridized for 30 minutes in Expresshyb (Clonetech). A CLZ_38 insert (25-100 ng) was labeled with [α-^{32}P]-d CTP by oligonucleotide labeling to specific activities of approximately $5x10^8$ cpm/μg and added to the prehybridization solution and incubated 1 hour. Filters were washed to high stringency (0.2 X SSC) (1 X SSC: 0.015 M NaCl and 0.0015 M Na citrate) at 68°C then exposed to Kodak X-AR film (Eastman Kodak, Rochester, NY) for up to 1 week. Figure 23 is a graphical representation of the described northern blot analyses. As shown, the pattern of CLZ_38 expression in clozapine-treated animals was similar to the pattern observed with TOGA analysis.

EXAMPLE 8
Characterization of CLZ 16

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-C-T-A-G; SEQ ID NO: 97) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel

electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_16 is slightly down-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_16 is an arm-repeat protein. In further characterization of CLZ_16, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_16 were performed to show the pattern of CLZ_16 mRNA expression in mouse anterior brain (24B) and posterior brain (24A). Control mice and mice treated with 7.5 mg/kg clozapine were sacrificed after two weeks. *In situ* hybridization was performed on free-floating sections (25 μM thick). Coronal sections were hybridized at 55°C for 16 hour with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_16 at 10^7 cpm/ml.

The probe was synthesized from the 3'-ended cDNA TOGA clone using the Maxiscript Transcription Kit (Ambion, Austin, TX). Excess probe was removed by washing with 2 X SSC (I X SSC = 0.015 M NaCl/0.0015 M Na citrate) containing 14 mM β-mercaptoethanol (30 minutes), followed by incubation with 4 μg/ml ribonuclease in 0.5 M NaCl/0.05 M EDTA/0.05 M Tris-HCl, pH 7.5, for 1 hour at 37° C. High stringency washes were carried out at 55°C for 2 hours in 0.5 X SSC/50% formamide/0.01 M β-mercaptoethanol, and then at 68°C for 1 hour in 0.1 X SSC/0.01 M β-mercaptoethanol/0.5% sarkosyl. Slices were mounted onto gelatin-coated slides and dehydrated with ethanol and chloroform before autoradiography. Slides were exposed for 1-4 days to Kodak X-AR film and then dipped in Ilford K-5 emulsion. After 4 weeks, slides were developed with Kodak D19 developer, fixed, and counterstained with Richardson's blue stain.

As shown in Figure 24A and B, CLZ_16 mRNA is expressed ubiquitously throughout mouse brain. Figure 24A shows dense labelling in the cortex and surrounding the hippocampal formation as well as moderate labelling in the dorsal thalamus and posterior brain. Figure 24B shows uniform labelling throughout.

EXAMPLE 9
Characterization of CLZ 17

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-C-T-C-A; SEQ ID NO: 99) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_17 is slightly down-regulated by clozapine treatment. Table 4 shows that CLZ_17 matches several ESTs isolated from mouse tissue. In further characterization of CLZ_17, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_17 were performed to show the pattern of CLZ_17 mRNA expression in mouse sections from anterior (25B) and posterior regions of the brain (25A).

In situ hybridization was performed on free-floating sections (25 μM thick) taken from control mice and mice treated with 7.5 mg/kg clozapine for 2 weeks. Coronal sections were hybridized at 55°C for 16 hour with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_17 at 10^7 cpm/ml. The probe was synthesized from the 3'-ended cDNA TOGA clone using the Maxiscript Transcription Kit (Ambion, Austin, TX). Excess probe was removed by washing as previously described in Example 8. Slices were mounted onto gelatin-coated slides and dehydrated with ethanol and chloroform before autoradiography. Slides were exposed for 1-4 days to Kodak X-AR film and then dipped in Ilford K-5 emulsion. After 4 weeks, slides were developed with Kodak D19 developer, fixed, and counterstained with Richardson's blue stain.

Figure 25A-B shows an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_17, showing the pattern of CLZ_17 mRNA expression in a coronal sections from posterior (25A) and anterior (25B)

regions of mouse brain. As shown, CLZ_17 mRNA is expressed in the cortex, hippocampus, striatum, and amygdala.

EXAMPLE 10
Characterization of CLZ 24

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-G-G-C-A; SEQ ID NO: 100) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_24 is up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_24 is an EST isolated from rat tissue. In further characterization of CLZ_24, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_24 were performed to show the pattern of CLZ_24 mRNA expression in mouse anterior brain (26B) and posterior brain (26A)

In situ hybridization was performed on free-floating sections (25 μM thick) obtained from comtrol mica nd mice treated with 7.5 mg/kg clozapine for 2 weeks. Coronal sections were hybridized at 55°C for 16 hour with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_24 at 10^7 cpm/ml. The probe was synthesized from the 3'-ended cDNA TOGA clone using the Maxiscript Transcription Kit (Ambion, Austin, TX). Excess probe was removed by washing as previously described in Example 8. Slices were mounted onto gelatin-coated slides and dehydrated with ethanol and chloroform before autoradiography. Slides were exposed for 1-4 days to Kodak X-AR film and then dipped in Ilford K-5 emulsion. After 4 weeks, slides were developed with Kodak D19 developer, fixed, and counterstained with Richardson's blue stain.

Figure 26A-B shows an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_24, showing the pattern of CLZ_24 mRNA expression in a coronal section through the hemispheres (26A) and cross section through the brainstem (26B) in mouse brain. As shown, CLZ_24 mRNA is ubiquitously expressed in the cortex.

EXAMPLE 11
Characterization of CLZ 26

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-G-G-C-T; SEQ ID NO: 101) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_26 is slightly down-regulated by clozapine treatment. Table 4 shows that CLZ_26 is a metalloprotease-disintegrin MDC15 mRNA. In further characterization of CLZ_26, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_26 were performed to show the pattern of CLZ_26 mRNA expression in mouse anterior brain (27B) and posterior brain (27A).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_26 using the methods described in the above examples.

Figure 27A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_26, showing the pattern of CLZ_26 mRNA expression in a coronal section of the hemispheres at the level of hippocampal

formation (27A) and coronal section of the hemispheres at the level of striatum (27B) in mouse brain. As shown, CLZ_26 mRNA is ubiquitously expressed in the cortex.

EXAMPLE 12
Characterization of CLZ 28

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-G-G-T-A; SEQ ID NO: 102) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_28 is down-regulated by clozapine treatment. Table 4 shows that CLZ_28 matches several ESTs isolated from mouse tissue. In further characterization of CLZ_28, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_28 were performed to show the pattern of CLZ_28 mRNA expression in mouse anterior brain (28B) and posterior brain (28A).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_28 using the methods described in the above examples.

Figure 28A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_28, showing the pattern of CLZ_28 mRNA expression in a coronal section through the hemispheres at the level of hippocampus (28A) and coronal section through the posterior region of hemispheres (28B) in mouse brain. As shown in Figure 28A and B, CLZ_28 mRNA is expressed ubiquitously in the cortex.

EXAMPLE 13
Characterization of CLZ 3

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-G-T-A; SEQ ID NO: 94) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_3 is up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_3 is a serine protease HTRA mRNA. In further characterization of CLZ_3, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_3 were performed to show the pattern of CLZ_3 mRNA expression in mouse anterior brain (29B) and posterior brain (29A).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_3 using the methods described in the above examples.

Figure 29A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_3, showing the pattern of CLZ_3 mRNA expression in a coronal section through the hemispheres at level of hippocampus (29A) and cross section through midbrain (29B) in mouse brain. As shown in Figure 29A and B, CLZ_3 mRNA is expressed in the cortex, thalamus, hippocampus, striatum, and amygdala.

EXAMPLE 14
Characterization of CLZ 34

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine

treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-A-T-T; SEQ ID NO: 103) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_34 is up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_34 is an N-methyl-D-aspartate receptor NMDAR1-2a subunit mRNA. In further characterization of CLZ_34, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_34 were performed to show the pattern of CLZ_34 mRNA expression in mouse anterior brain (30B) and posterior brain (30A).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_34 using the methods described in the above examples.

Figure 30A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_34, showing the pattern of CLZ_34 mRNA expression in a coronal section through the hemispheres at the level of hippocampus (30A) and cross section through the midbrain (30B) in mouse brain. As shown in Figure 30A and B, CLZ_34 mRNA is ubiquitously expressed.

EXAMPLE 15
Characterization of CLZ 43

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-C-T-A-A; SEQ ID NO: 104) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel

electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_43 is up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_43 matches an EST isolated from mouse tissue that matches oxysterol binding protein family member. In further characterization of CLZ_43, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_43 were performed to show the pattern of CLZ_43 mRNA expression in mouse anterior brain (31C), midbrain (31A), and posterior brain (31B).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_43 using the methods described in the above examples.

Figure 31A-C is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_43, showing the pattern of CLZ_43 mRNA expression in coronal sections of the hemispheres showing in the cortex, and intense lebelling in the striatum (31A-C) in mouse brain. Comparison with brain sections obtained from control mice showed that CLZ_43 expression is increased approximately 10-fold by chronic treatment (2 weeks) with clozapine.

Following the cloning of the mouse DST CLZ_43, a BLAST analysis was performed. A human homology was identified as a 5556 b.p. GenBank entry (AB040884, also known as KIAA1451). An oligonucleotide was chosen from this sequence and used to isolate the remaining 5' end of the human gene from an adult human brain cDNA plasmid library. Using the method described below, a 1717 b.p. cDNA clone (SEQ ID NO:103) was isolated that overlaps the human sequence. This clone provides an additional (novel) 512 b.p. at the 5' end of the GenBank entry. Sequence analysis suggests the position of the methionine start codon for the open reading frame is at base 562 of the 1717 b.p. clone (SEQ ID NO: 108). The open reading frame of the 1717 b.p. clone encodes a 385 amino acid peptide (SEQ ID NO: 108, SEQ ID NO: 109).

The following methods were used to isolate the 1717 b.p. cDNA clone. The target pool was a cDNA plasmid library created from adult human brain RNA. The oligonucleotide sequence used for hybridization was 5' - AAC AAG TCC GTC CTG GCA TGG-3' (SEQ ID NO:88). The clone was isolated using the methods prescribed by the manufacturer of the GeneTrapper kit (Life Technologies, Inc.). Capture oligonucleotide were prepared by end-labeling the oligonucleotide with biotin-14-dCTP using terminal deoxynucloetidyl transferase. The cDNA plasmid pool was converted from double-stranded cDNA to single-stranded cDNA through the specific action of GeneII protein and exonuclease III. The single-stranded cDNA pool was combined with the end-labelled oligonucleotide and hybridization was allowed to occur at room temperature for 30 minutes. The reaction was then mixed with strepavidin-coated magnetic beads. The single-stranded cDNA plasmids that hybridized to the oligonucleotide were purified using a magnet to retain the magnetic beads in the reaction tube while all of the unbound components were washed away. The single-stranded plasmid DNA was released from the oligonucleotide and repaired back into a double-stranded plasmid using a fresh sample of the capture oligonucleotide and DNA polymerase. The repaired plasmids were transformed into bacteria and plated on an agar plate. The following day, bacterial colonies were individually picked and grown overnight. Plasmid DNA was prepared from these mini-preparations and subjected to sequence analysis.

Homology matches with a human genome database have identified 7 exons spread across more than 22,000 b.p. Further it has been determined that CLZ_43 maps to chromosome 12, which is not a chromosome previously linked to schizophrenia. The sequence data reveals that the open reading frame encodes a protein of 472 amino acids (SEQ ID NO: 110). Comparison with protein databases indicate that the protein is novel and is a member of a class of proteins that binds lipids, especially oxysterols.

The observation that, of thousands of proteins expressed by the striatum, apoD and a novel oxysterol binding protein are among the few modulated by neuroleptic drugs strengthens the hypothesis that schizophrenia is a disease of brain sterol homeostasis, and thus may have etiologies as diverse as atherosclerosis. The brain has by far more cholesterol and 24S-hydroxysterol than any organ other than the

adrenal glands, and the special importance of the membrane activities of neurons and their myelinating cells are self-evident. The lipid bilayer of the membrane is made up of glycerolphopholipids and cholesterol, and variations in composition and hydrocarbon chain saturation state determine membrane order and fluidity. These properties affect the binding of extrinsic membrane proteins and, thus, second messenger signaling. As we have shown previously, a large percentage of the mRNAs highly enriched in the striatum encode proteins that regulate second messenger signaling along the inner membrane. Thus, a panneural or panorganismic disruption in lipid metabolism might manifest first as a striatal disease. As of now, this is a somewhat impressionistic concept. Working out the nature of the neuroleptic drug effects on membrane properties may bring the issue into greater focus.

EXAMPLE 16
Characterization of CLZ 44

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-A-C-G-G; SEQ ID NO: 105) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_44 is up-regulated by clozapine treatment. Tables 2 and 3 show that CLZ_44 matches an EST isolated from mouse tissue. In further characterization of CLZ_44, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_44 were performed to show the pattern of CLZ_44 mRNA expression in mouse anterior brain (32A) and posterior brain (32B).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_44 using the methods described in the above examples.

Figure 32A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_44, showing the pattern of CLZ_44 mRNA expression in a coronal section showing labelling in the hippocampus, hypothalamus, and temporal cortex (32A) and coronal section showing cortical labelling (32B) in mouse brain.

EXAMPLE 17
Characterization of CLZ 64

Male C57Bl/6J mice (20-28 g) were housed as previously described in Example 1. The same experimental paradigm used in Example 1 for clozapine treatment was used for the TOGA analyses. The TOGA data was generated with a 5'-PCR primer (C-G-A-C-G-G-T-A-T-C-G-G-T-C-A-T; SEQ ID NO: 106) paired with the "universal" 3' primer (SEQ ID NO: 23) labeled with 6-carboxyfluorescein (6FAM, ABI) at the 5' terminus. PCR reaction products were resolved by gel electrophoresis on 4.5% acrylamide gels and fluorescence data acquired on ABI377 automated sequencers. Data were analyzed using GeneScan software (Perkin-Elmer).

As shown in Table 1, the results of TOGA analysis indicate that CLZ_64 is up-regulated by chronic clozapine treatment. Tables 2 and 3 show that CLZ_64 matches an EST isolated from mouse tissue and shares homolgy with mitochondrial enoyl-CoA hydratase mRNA. In further characterization of CLZ_64, *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_64 were performed to show the pattern of CLZ_64 mRNA expression in mouse anterior brain (33B) and mid-brain (33A).

In situ hybridization was performed on free-floating coronal sections (25 μM thick) with an ^{35}S-labeled, single-stranded antisense cRNA probe of CLZ_64 using the methods described in the above examples.

Figure 33A-B is an *in situ* hybridization analysis using an antisense cRNA probe directed against the 3' end of CLZ_64, showing the pattern of CLZ_64 mRNA expression in different coronal sections of the hemispheres in mouse brain. As shown in Figure 33A and B, CLZ_64 mRNA is ubiquitously expressed.

We claim:

1. An isolated nucleic acid molecule comprising a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107.

2. An isolated polypeptide encoded by a polynucleotide chosen from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO: 49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO: 57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72 and SEQ ID NO:107.

3. An isolated polypeptide of SEQ ID NO:109.

4. An isolated polypeptide of SEQ ID NO:110.

5. An isolated nucleic acid molecule comprising a polynucleotide at least 95% identical to the isolated nucleic acid molecule of claim 1.

6. An isolated nucleic acid molecule at least ten bases in length that is hybridizable to the isolated nucleic acid molecule of claim 1 under stringent conditions.

7. An isolated nucleic acid molecule encoding the polypeptide of claim 2.

8. An isolated nucleic acid molecule encoding a fragment of the polypeptide of claim 2.

9. An isolated nucleic acid molecule encoding a polypeptide epitope of the polypeptide of claim 2.

10. The polypeptide of claim 2 wherein the polypeptide has biological activity.

11. An isolated nucleic acid encoding a species homologue of the polypeptide of claim 2.

12. The isolated nucleic acid molecule of claim 1, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the 5' end or the 3'end.

13. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

14. A recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

15. A method of making the recombinant host cell of claim 14.

16. The recombinant host cell of claim 14 comprising vector sequences.

17. The isolated polypeptide of claim 2, wherein the isolated polypeptide comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

18. An isolated antibody that binds specifically to the isolated polypeptide of claim 2.

19. An isolated antibody that binds specifically to the isolated polypeptide of claim 3.

20. An isolated antibody that binds specifically to the isolated polypeptide of claim 4.

21. The isolated antibody of claims 16, 17 or 18 wherein the antibody is a monoclonal antibody.

22. The isolated antibody of claims 16, 17 or 18 wherein the antibody is a polyclonal antibody.

23. A recombinant host cell that expresses the isolated polypeptides of claim 2, 3 or 4.

24. An isolated polypeptide produced by the steps of:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed; and

(b) isolating the polypeptide.

25. A method for preventing, treating, modulating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claims 2, 3 or 4, or the polynucleotide of claim 1.

26. The method of claim 25 wherein the medical condition is a neuropsychiatric disorder.

27. A method for preventing, treating, modulating, or ameliorating a medical condition comprising administering to a mammalian subject a therapeutically effective amount of the antibody of claims 18, 19 or 20.

28. The method of claim 27 wherein the medical condition is a neuropsychiatric disorder.

29. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

30. The method of claim 29 wherein the pathological condition is a neuropsychiatric disorder.

30. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising detecting an alteration in expression of a polypeptide encoded by the polynucleotide of claim 1, wherein the presence of an alteration in expression of the polypeptide is indicative of the pathological condition or susceptibility to the pathological condition.

31. The method of claim 30 wherein the alteration in expression is an increase in the amount of expression or a decrease in the amount of expression.

32. The method of claim 30 wherein the pathological condition is a neuropsychiatric disorder.

33. The method of claim 32 wherein the method further comprises the steps of: obtaining a first biological sample from a patient suspected of having a neuropsychiatric disorder and obtaining a second sample from a suitable comparable control source;

(a) determining the amount of at least one polypeptide encoded by a polynucleotide of claim 1in the first and second sample; and

(b) comparing the amount of the polypeptide in the first and second samples;

wherein a patient is diagnosed as having a neuropsychiatric disorder if the amount of the polypeptide in the first sample is greater than or less than the amount of the polypeptide in the second sample.

34. The use of the polynucleotide of claim 1 or polypeptide of claims 2, 3 or 4 for the manufacture of a medicament for the treatment of a neuropsychiatric disorder.

35. The use of the antibody of claims 18, 19 or 20 for the manufacture of a medicament for the treatment of a neuropsychiatric disorder.

36. A method for identifying a binding partner to the polypeptide of claims 2, 3 or 4 comprising:

(a) contacting the polypeptide of claim 2, 3 or 4 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

37. The gene corresponding to the cDNA sequence of the isolated nucleic acid of claim 1.

38. A method of identifying an activity of an expressed polypeptide in a biological assay, wherein the method comprises:

(a) expressing the polypeptide of claims 2, 3 or 4 in a cell;

(b) isolating the expressed polypeptide;

(c) testing the expressed polypeptide for an activity in a biological assay; and

(d) identifying the activity of the expressed polypeptide based on the test results.

39. A substantially pure isolated DNA molecule suitable for use as a probe for genes regulated by neuroleptics, chosen from the group consisting of the DNA molecules identified in Table 1, having a 5' partial nucleotide sequence and length as described by their digital address, and having a characteristic regulation pattern by neuroleptics.

40. A kit for detecting the presence of the polypeptide of the claims 2, 3 or 4 in a mammalian tissue sample comprising a first antibody which immunoreacts with a mammalian protein encoded by a gene corresponding to the polynucleotide of claim 1 or with a polypeptide encoded by the polynucleotide of claim 2, 3 or 4 in an amount sufficient for at least one assay and suitable packaging material.

41. A kit of claim 40 further comprising a second antibody that binds to the first antibody.

42. The kit of claim 41 wherein the second antibody is labeled.

43. The kit of claim 42 wherein the label comprises enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, or bioluminescent compounds.

44. A kit for detecting the presence of a genes encoding an protein comprising a polynucleotide of claim 1, or fragment thereof having at least 10 contiguous bases, in an amount sufficient for at least one assay, and suitable packaging material.

45. A method for detecting the presence of a nucleic acid encoding a protein in a mammalian tissue sample, comprising the steps of:

(a) hybridizing a polynucleotide of claim 1 or fragment thereof having at least 10 contiguous bases, with the nucleic acid of the sample; and

(b) detecting the presence of the hybridization product.

46. A method of diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder in a subject comprising:

(a) determining the presence or absence of a mutation in apolipoprotein D polynucleotide; and

(b) diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder based on the presence or absence of said mutation.

47. A method of diagnosing a neuropsychiatric disorder or a susceptibility to a neuropyschiatric disorder in a subject comprising:

(a) determining the presence or amount of expression of apolipoprotein D polypeptide in a biological sample; and

(b) diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder based on the presence or amount of expression of the apolipoprotein D polypeptide.

48. The method of claims 46 or 47 wherein the neuropsychiatric disorder is schizophrenia.

49. The method of claims 46 or 47 wherein the neuropsychiatric disorder is bipolar disorder.

50. A method of diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide or polynucleotide fragment of SEQ ID NO: 2 and

(b) diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder based on the presence or absence of said mutation.

51. A method of diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide comprising an amino acid sequence at least 95% identical to a polypeptide fragment of a translation of SEQ ID NO: 2 in a biological sample; and

(b) diagnosing a neuropsychiatric disorder or a susceptibility to a neuropsychiatric disorder based on the presence or amount of expression of the polypeptide.

52. The method of claims 50 or 51 wherein the neuropsychiatric disorder is schizophrenia.

53. The method of claims 50 or 51 wherein the neuropsychiatric disorder is bipolar disorder.

54. The method of claims 50 or 51wherein the neuropsychiatric disorder is addiction-related behavior.

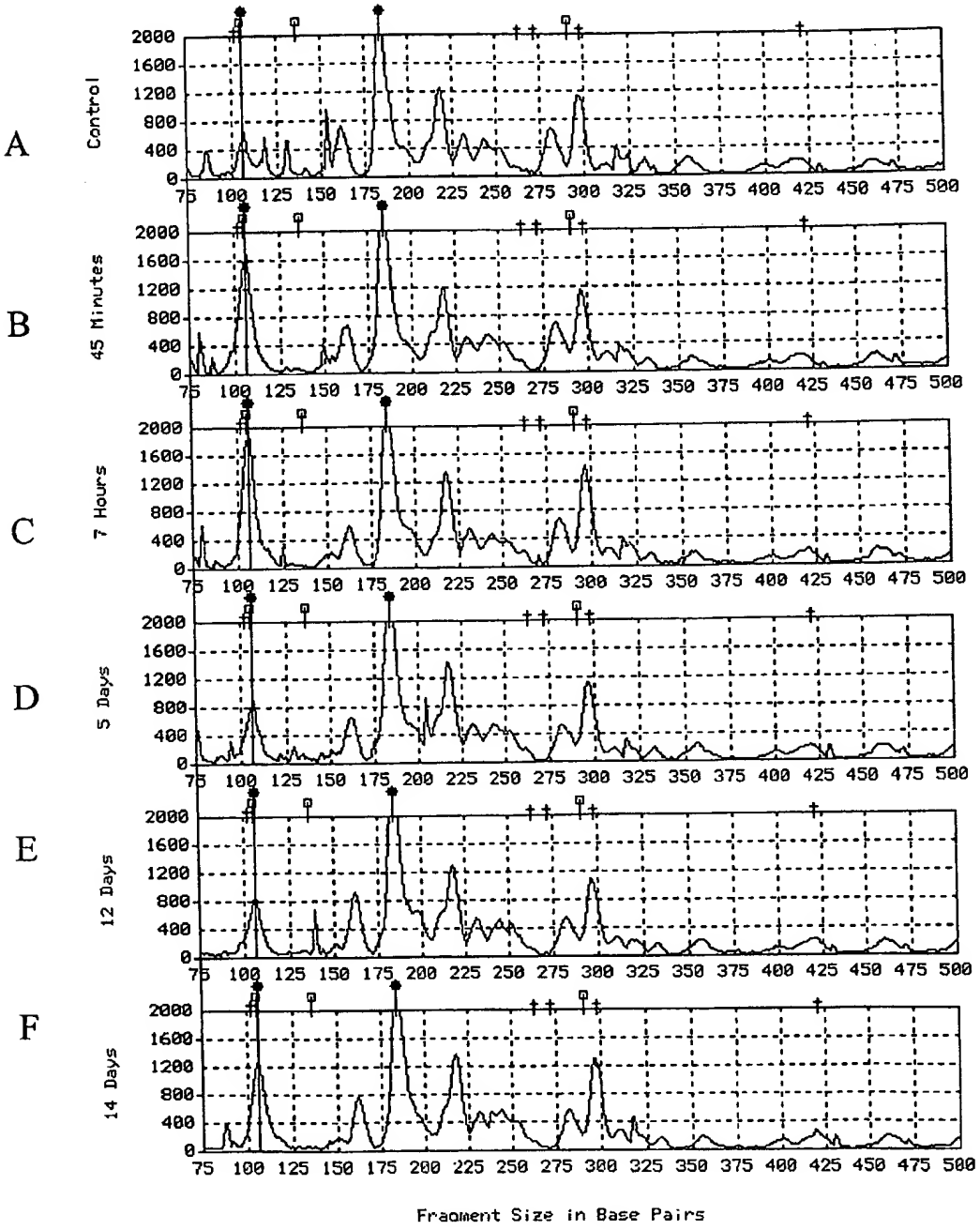
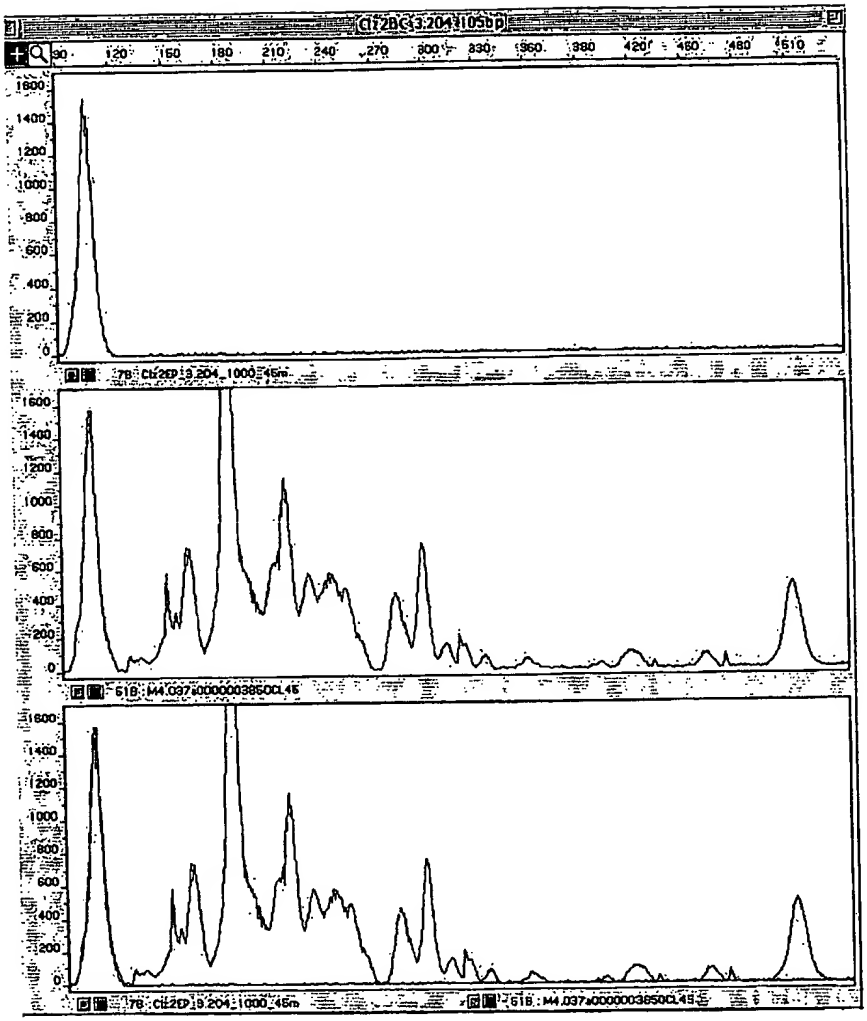

A
B
C
D
E
F
Control
45 Minutes
7 Hours
5 Days
12 Days
14 Days
2000
1600
1200
800
400
0
75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Fragment Size in Base Pairs

Fig. 1

A

B

C



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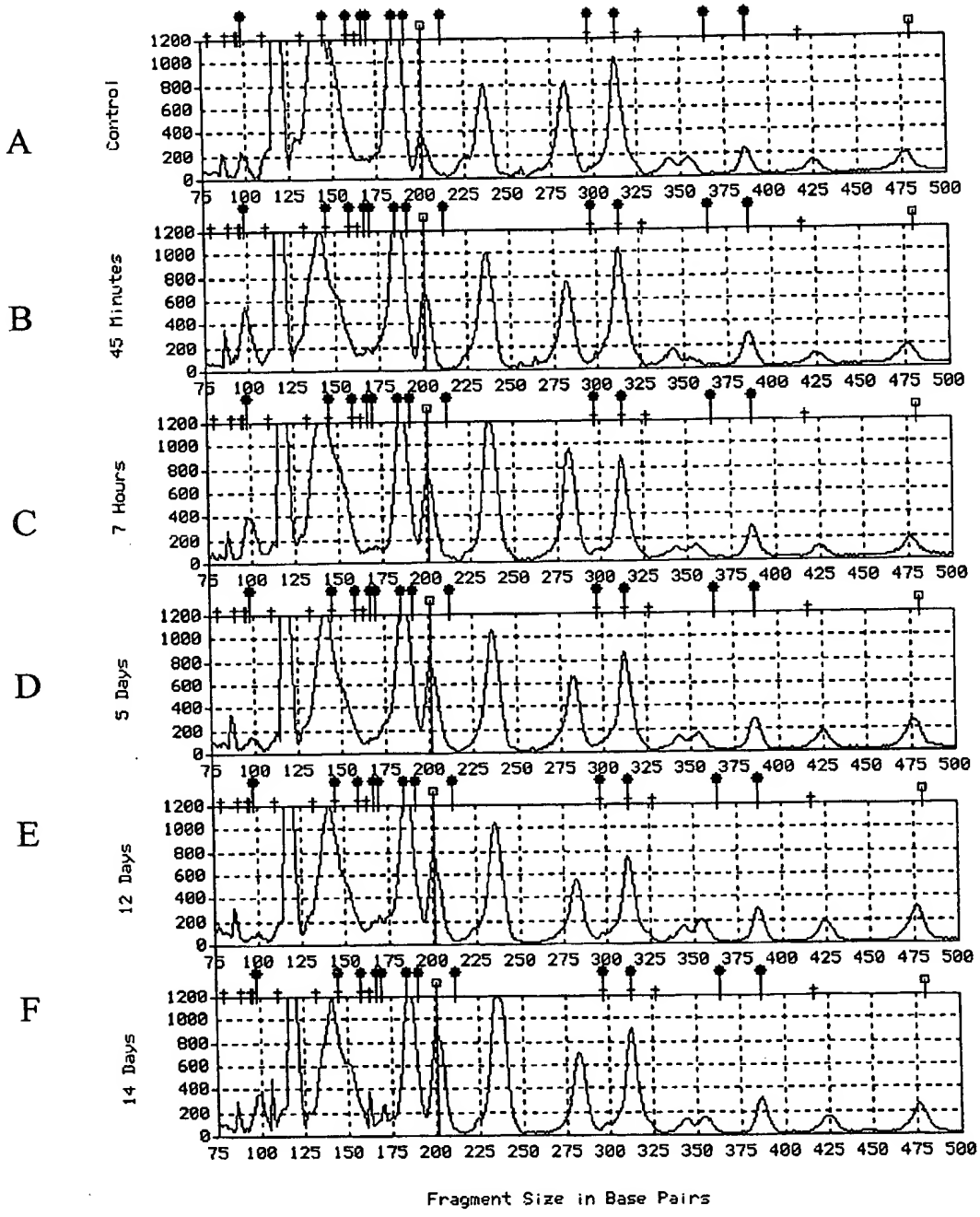

A
B
C
D
E
F
Control
45 Minutes
7 Hours
5 Days
12 Days
14 Days
1200
1000
800
600
400
200
0
75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Fragment Size in Base Pairs

Fig. 3

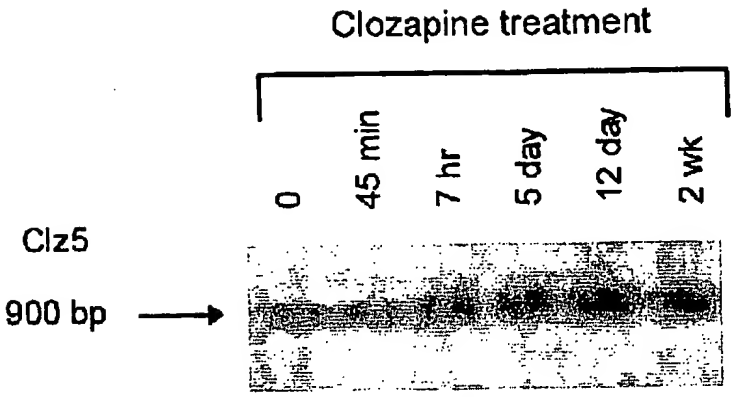

Clozapine treatment
0
45 min
7 hr
5 day
12 day
2 wk
Clz5
900 bp

Fig. 4

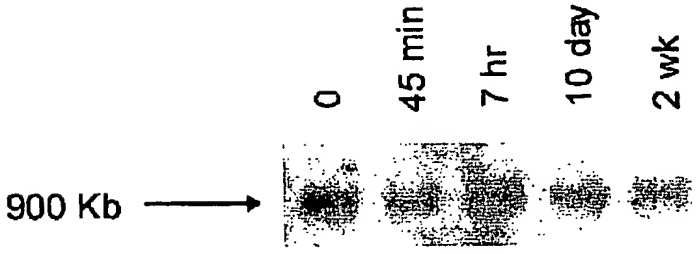

0
45 min
7 hr
10 day
2 wk
900 Kb

Fig. 5

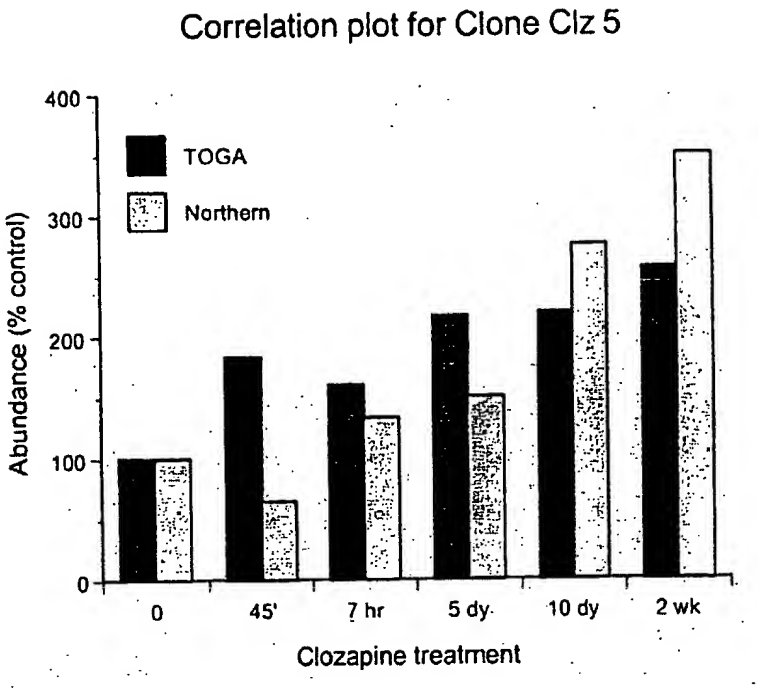
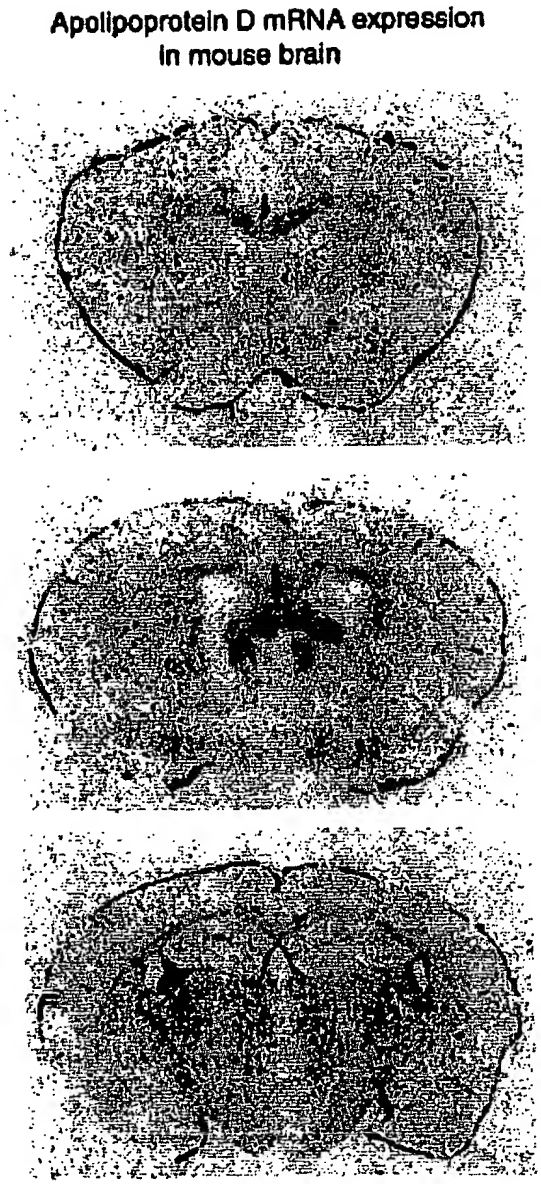

Correlation plot for Clone Clz 5
400
300
200
100
0
Abundance (% control)
TOGA
Northern
0
45'
7 hr
5 dy
10 dy
2 wk
Clozapine treatment

Fig. 6



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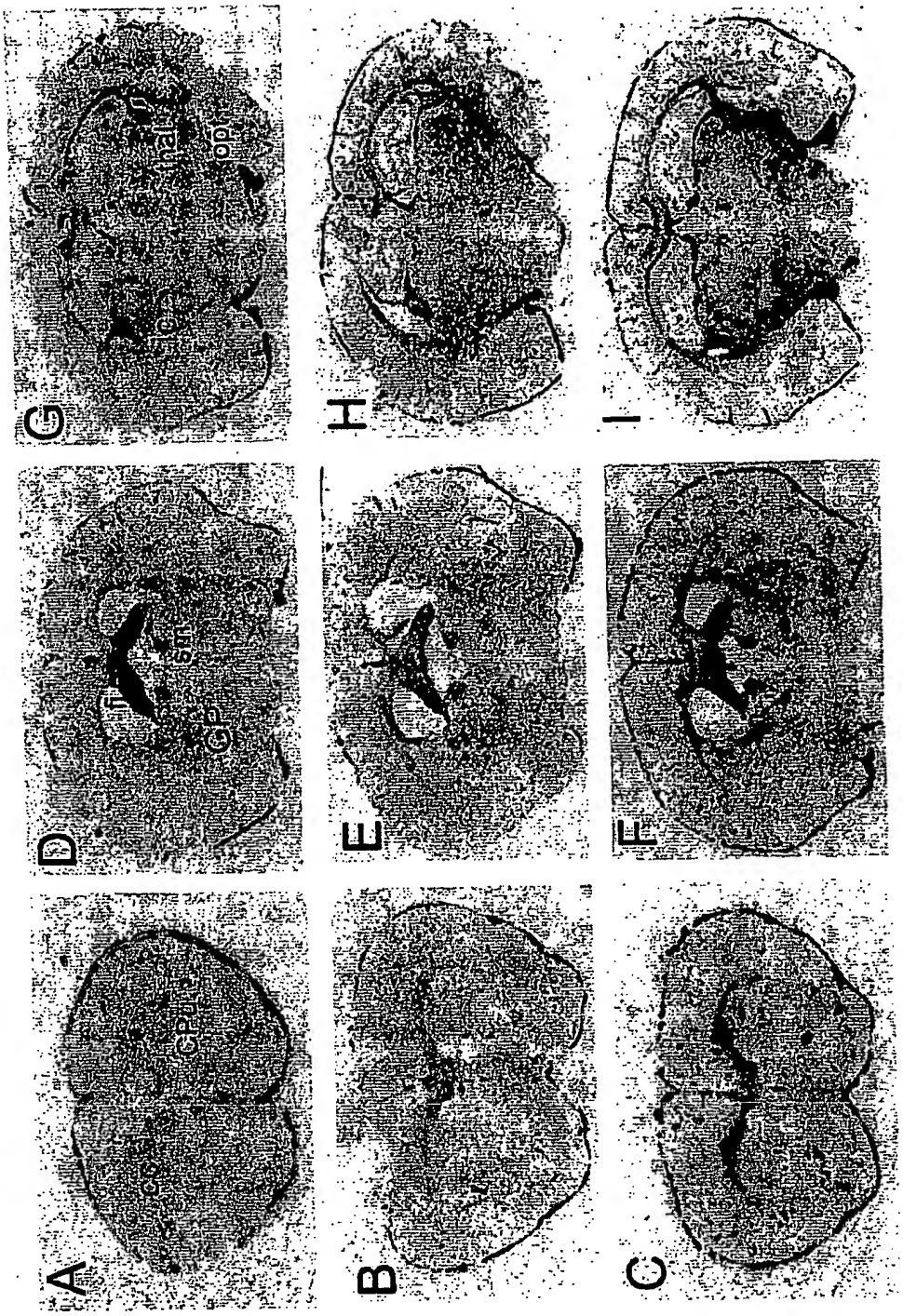

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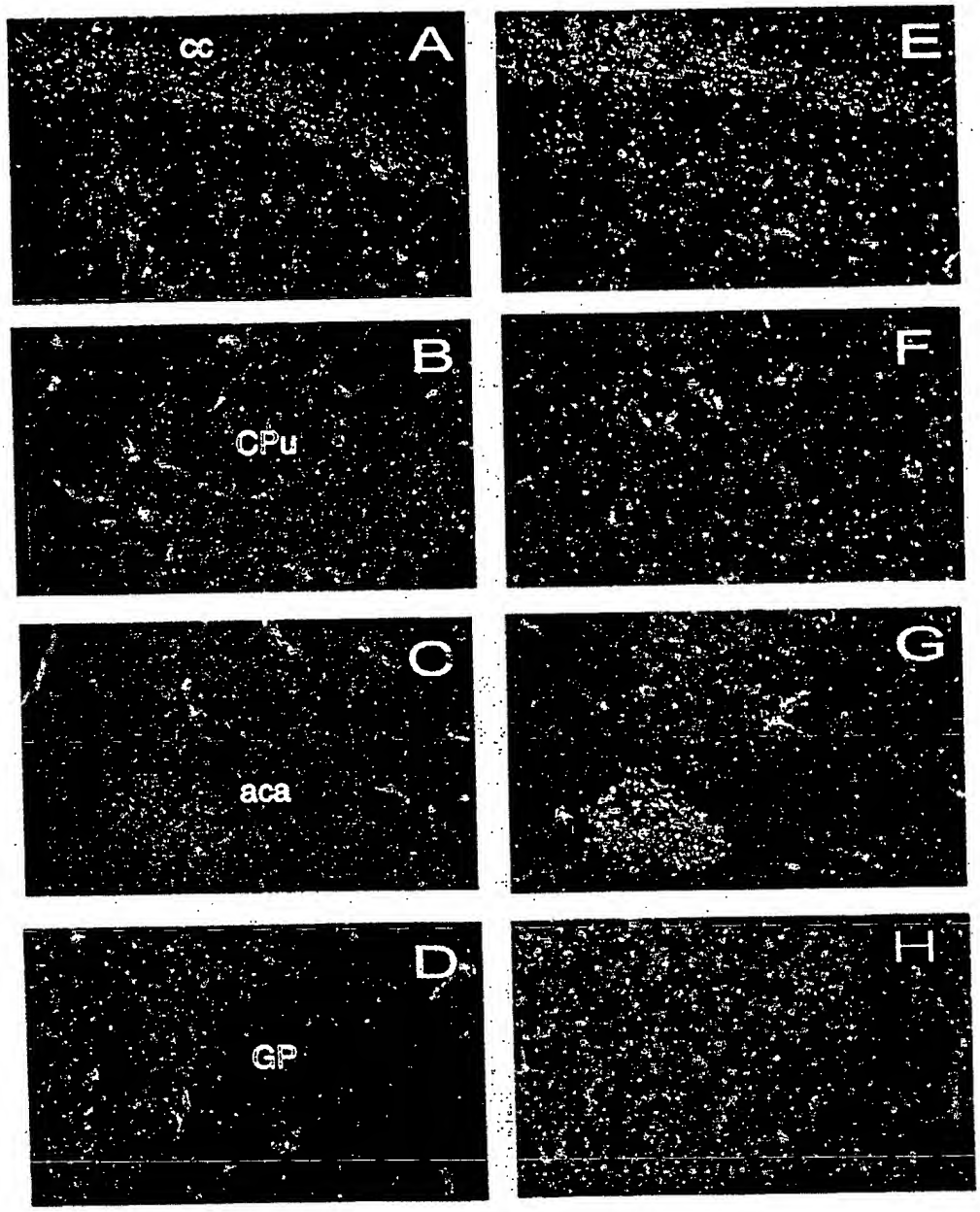
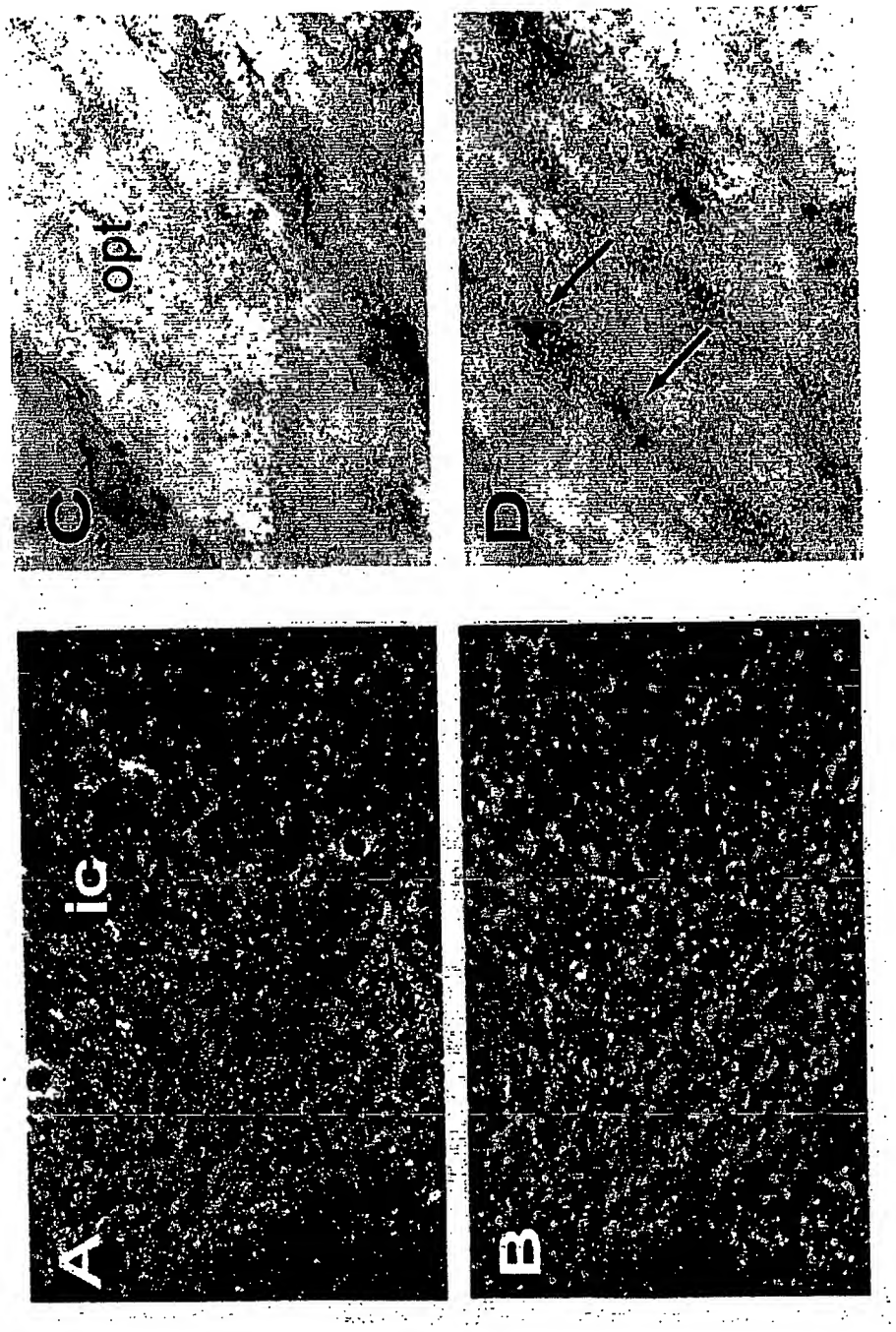


Fig. 9



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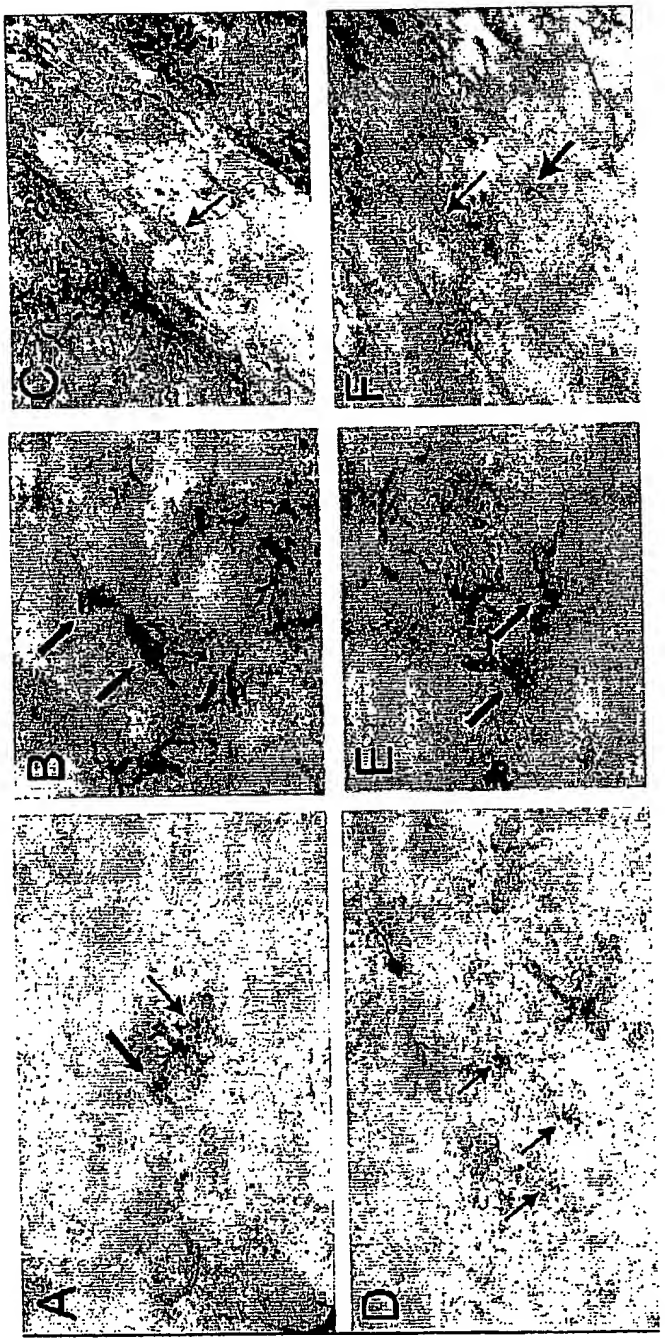


Fig. 11

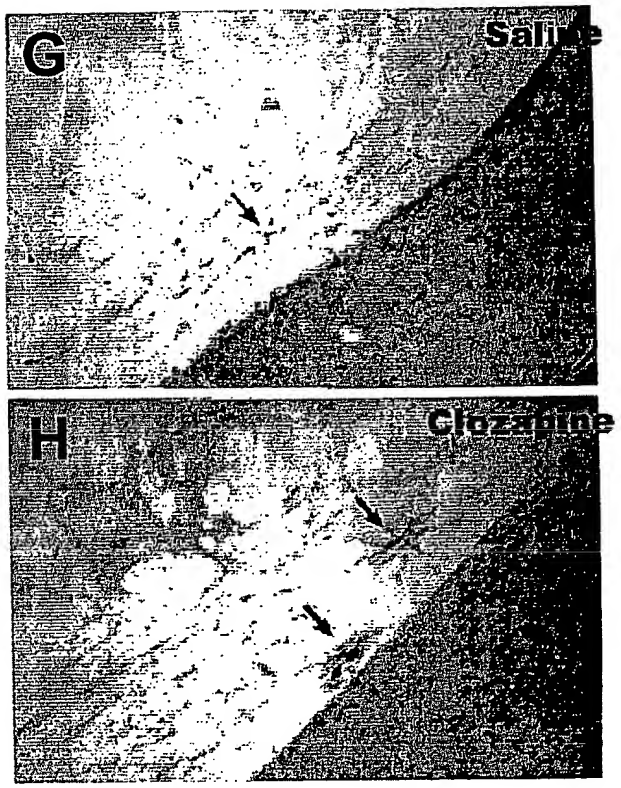
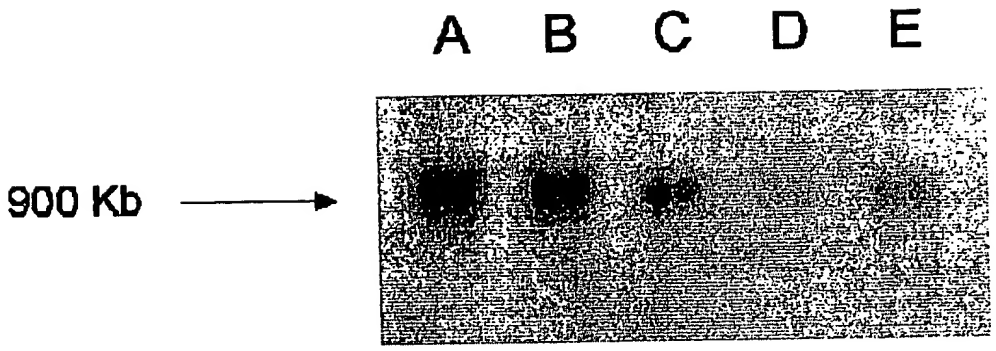


Fig. 11 (con't)



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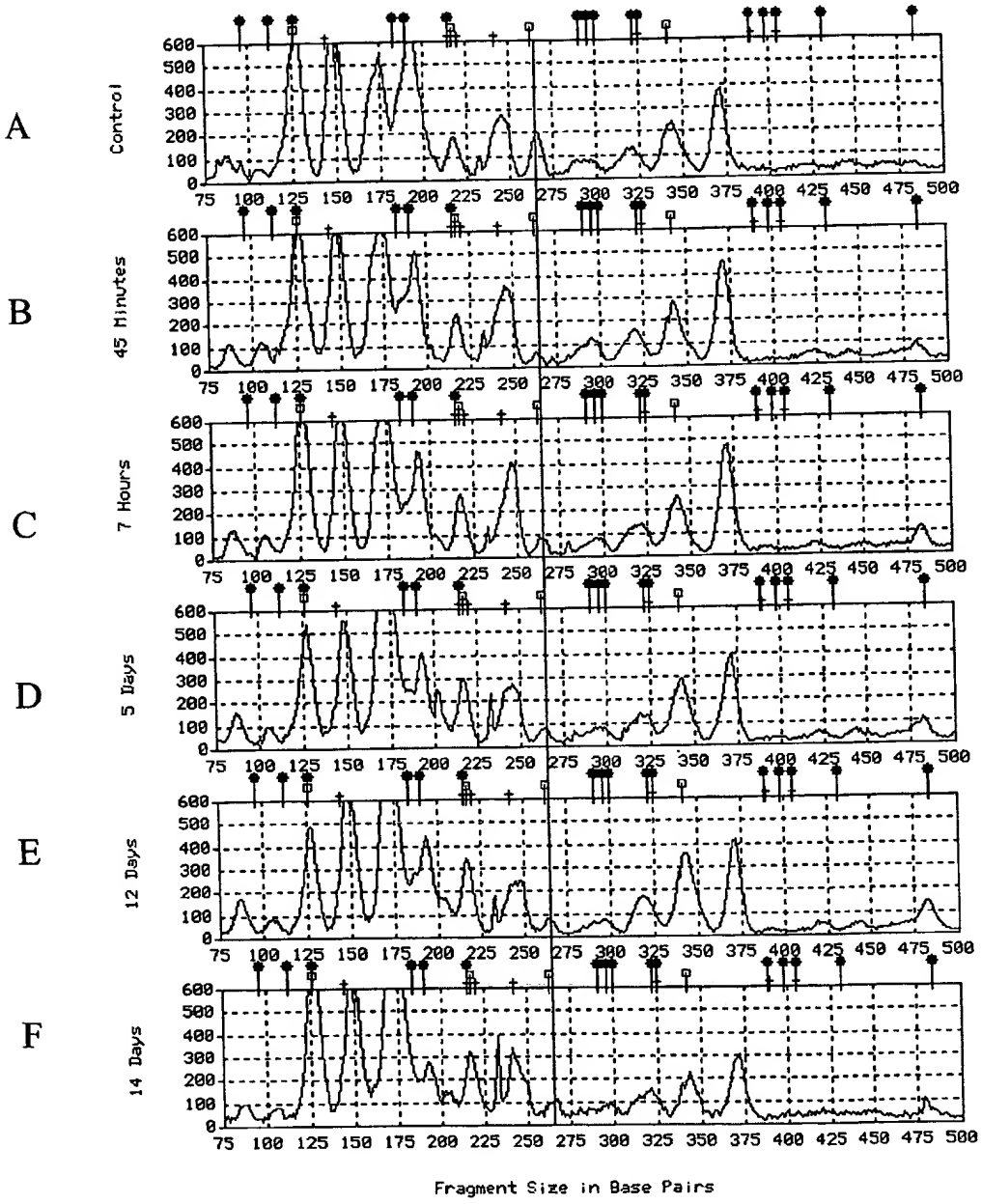
A
B
C
D
E
F
Control
45 Minutes
7 Hours
5 Days
12 Days
14 Days
600
500
400
300
200
100
0
75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Fragment Size in Base Pairs

Fig. 13

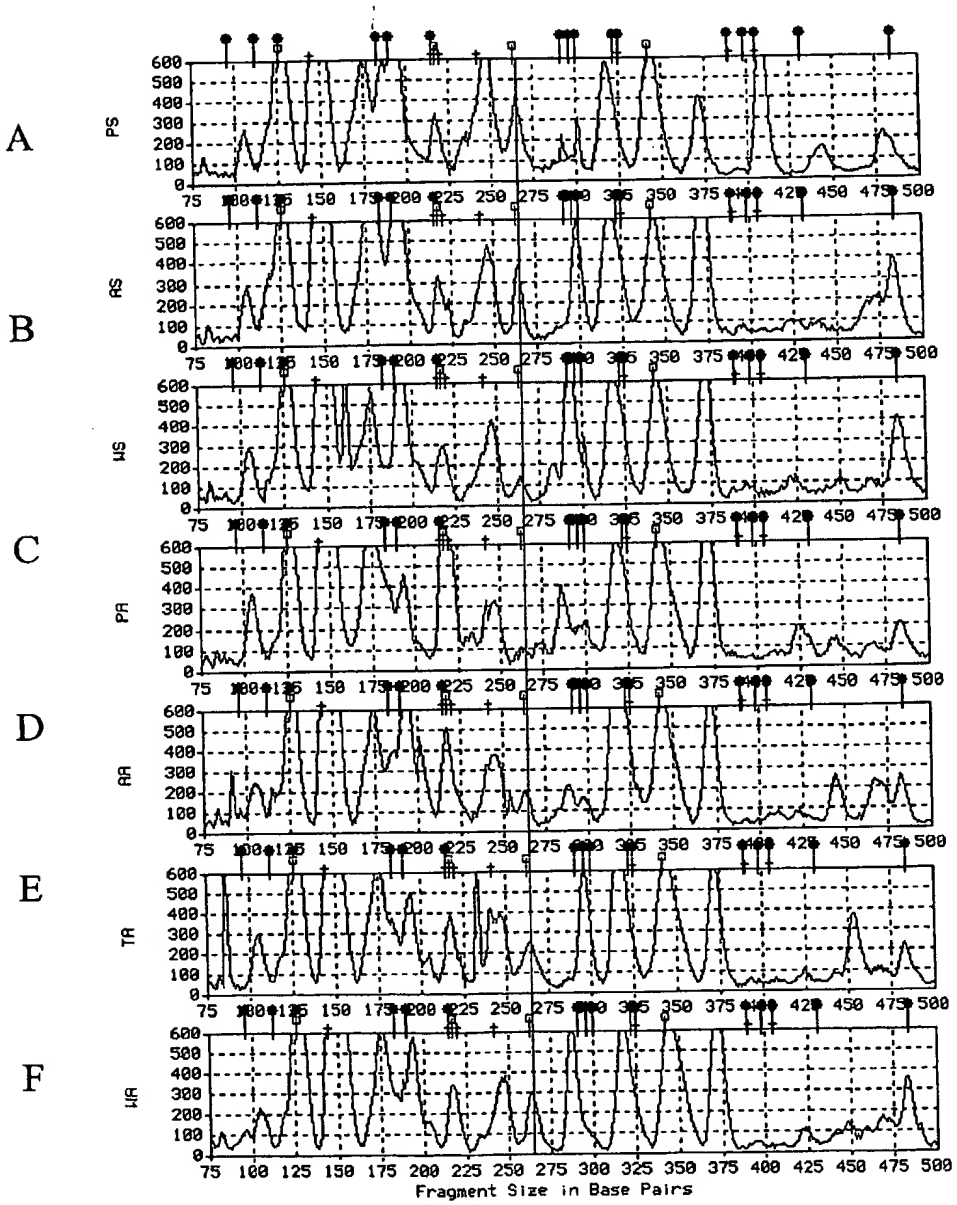
A
B
C
D
E
F
PS
AS
SM
PA
AA
TA
WA
600
500
400
300
200
100
0
75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Fragment Size in Base Pairs

Fig. 14

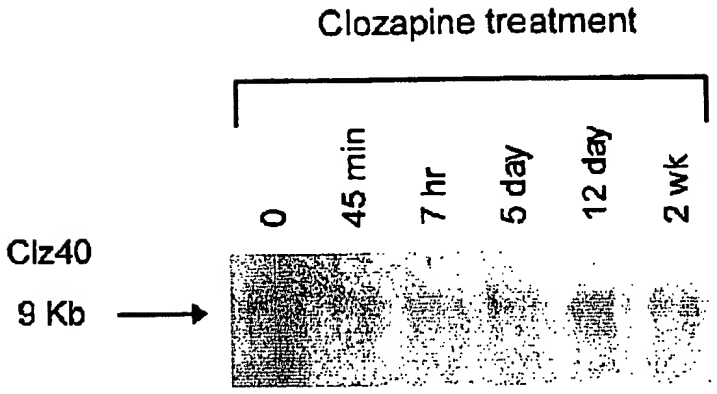
Clozapine treatment
0
45 min
7 hr
5 day
12 day
2 wk
Clz40
9 Kb

Fig. 15

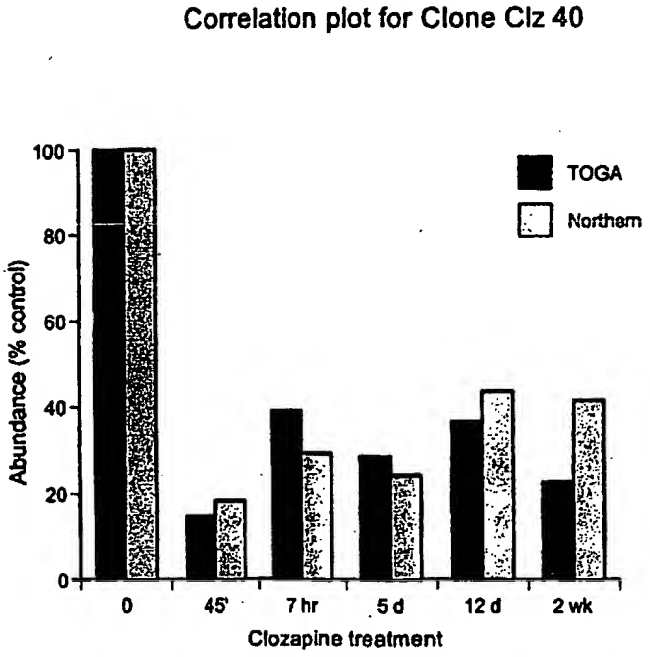
Correlation plot for Clone Clz 40
TOGA
Northern
Abundance (% control)
100
80
60
40
20
0
0
45'
7 hr
5 d
12 d
2 wk
Clozapine treatment

Fig. 16

A

B

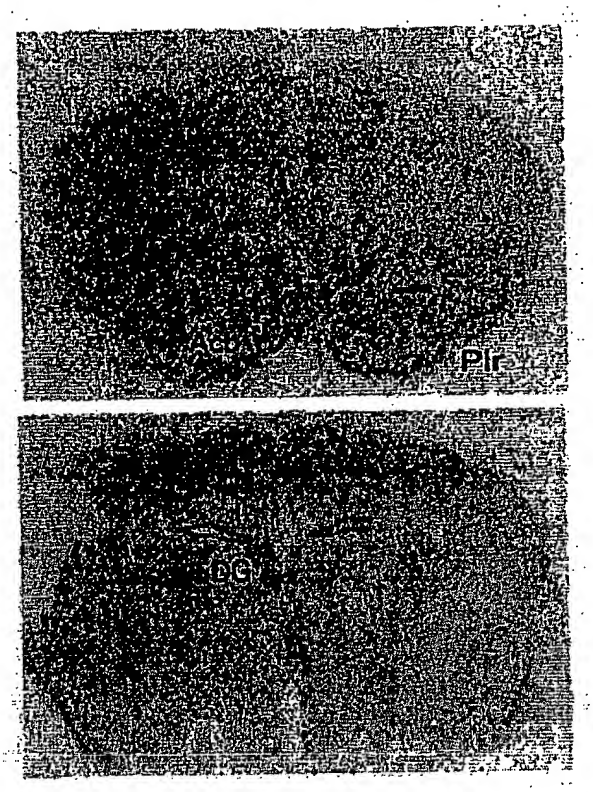

Fig. 17

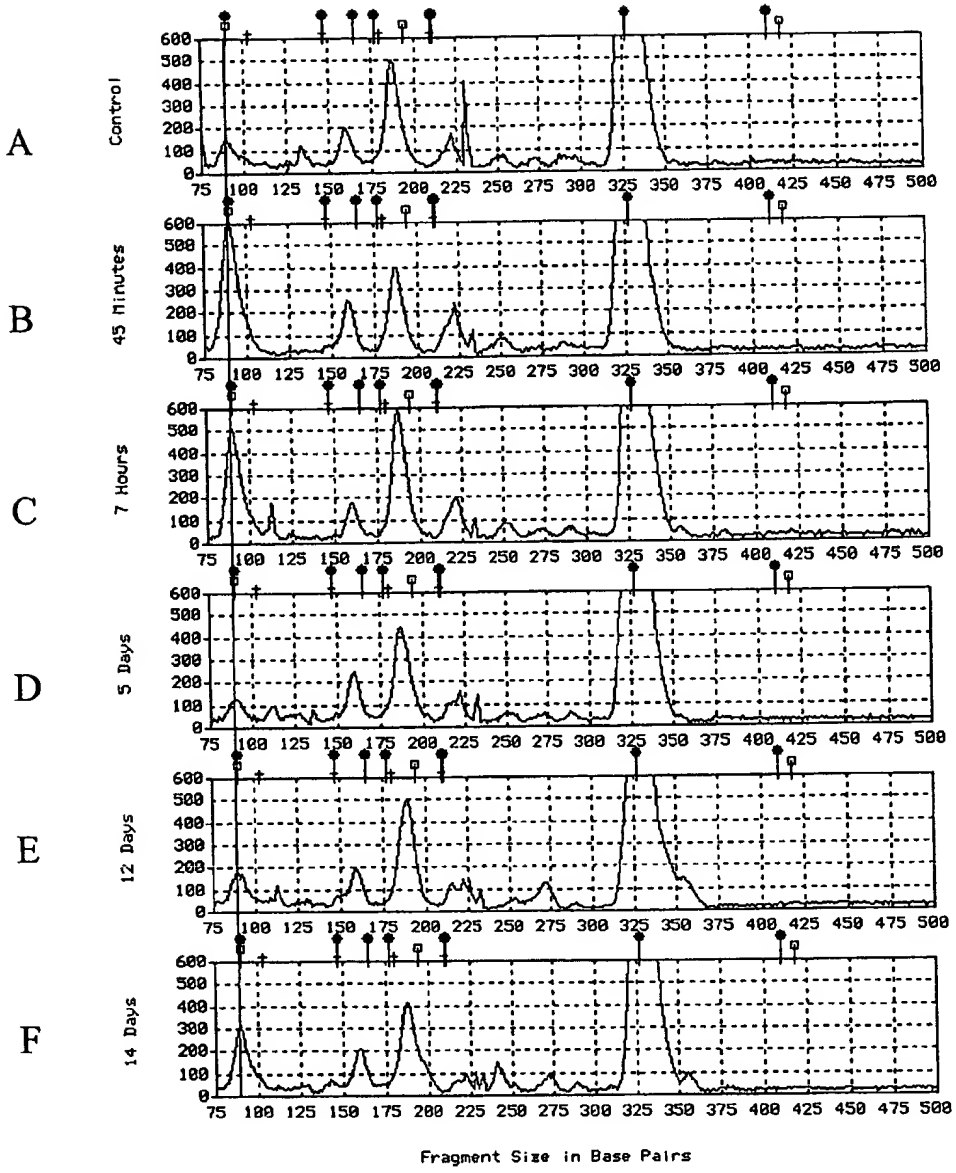
A
B
C
D
E
F
Control
45 Minutes
7 Hours
5 Days
12 Days
14 Days
0
100
200
300
400
500
600
75 100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500
Fragment Size in Base Pairs

Fig.18

```
1    CAGTTTCCCT GCACCTCTGG CTCTCACCAG CATCCTCTCA GCTGTTCTGT GCCAAATACG
61   TCTGCACCCA TGGGTTCAGC ACAGGGGCCC CTCACCTCGG TGACTAAGCT TCGCCCACCT
121  TGTTACGATG TCTTATATAT ATACACACTG CTATTTACAG ACCTGGCCTT GGATCCTGTG
181  ACCCTCTGGG AAGAGTCCTG CCAAAGTCCA GGACAGCATT GGGGCTAAGG CAGAGGCTTT
241  TCTCTAGAGG CTTGGGCCCT GTCCCAACGT GGACTTTGGG GCTAGGAACC TGGGCTTCTC
301  TCTGTGAATG TAAGGACAGC TACTCAGAGA GCCTTGATGG GGGCCTCTCC CCATTTCCTG
361  TGGTCAGACC CGGCTCAGCA CTTGGCAGCT GTCCCGTGCG GGGACTCAGT CCAACTCTGT
421  GTTTGCTTTT CTTCTTGGCC AAAGCATGTG CCACTAAGCT GTCCTGGAGG ACATTGTCTT
481  TATGAAACAC ACCTGGAATA AAACCACTTC TTACATGTCC AAAANAAAAA AAAAAAA
```

Fig. 19

```
   1  AGCTGGTGTC CGGCGGGGCC GTGGCTGCTC CTCCACGCGT AGCCCCGCAC
  51  CTGCTGCCCC AGTCCAGCCC GGAGCTCCGC GGCCATGCGG CTGGCGCTGC
 101  TCTGGGCTCT GGGACTCCTG GGCGCGGGCA GCCCTCGGCC CTCCCCGCCG
 151  CTGCCAAATA TAGGAGGCAC TGAGGAAGAG CAGCAAGCCA GCCCAGAGAG
 201  GACGCTGAGT GGATCCATGG AGAGCCGGGT TGTTCAGGAC AGCCCCCCAA
 251  TGAGCCTAGC AGACGTGCTT CAGACTGGTT TACCTGAGGC CCTGAGGATT
 301  TCCTTGGAGC TGGACAGTGA GAGTCATGTC CTGGAGCTTC TACAAAATAG
 351  AGATCTAATC CCTGGCCGCC CAACTCTGGT GTGGTACCAG CCTGATGGCA
 401  CCCGAATGGT CAGCGAGGGC TACAGTCTAG AAAACTGCTG CTACCGAGGA
 451  CGAGTGCAGG GCCACCCCAG CTCCTGGGTG TCCCTCTGTG CCTGCTCTGG
 501  GATCAGGGGG CTCATTGTCC TGTCCCCAGA GAGAGGCTAT ACACTGGAGC
 551  TGGGCCCTGG GGACCTTCAG CGTCCTGTCA TTTCTCGGAT CCAAGACCAC
 601  CTGTTGCTGG GCCACACCTG TGCCCCAAGC TGGCATGCCT CTGTGCCCAC
 651  TCGGGCAGGA CCAGACCTCC TTCTGGAACA GCATCACGCT CACAGGCTTA
 701  AGCGAGATGT AGTAACAGAG ACGAAAATTG TGGAGTTGGT GATTGTGGCT
 751  GATAATTCAG AGGTCAGAAA GTACCCTGAC TTCCAACAAC TGCTGAACCG
 801  GACACTAGAA GCGGCTCTCT TGCTAGACAC GTTCTTCCAG CCCTTGAATG
 851  TCCGGGTAGC CCTTGTGGGC CTAGAGGCAT GGACCCAGCA CAACCTGATA
 901  GAAATGAGCT CCAACCCAGC TGTCCTGCTA GACAACTTCC TCCGCTGGCG
 951  CCGGACAGAC TTGCTGCCTC GACTGCCCCA TGACAGTGCC CAACTGGTGA
1001  CTGTAACTTC CTTCTCTGGT CCCATGGTGG GCATGGCCAT TCAGAATTCC
1051  ATCTGTTCCC CTGACTTCTC CGGAGGTGTG AATATGGACC ACTCCACAAG
1101  CATCTTAGGC GTTGCCTCCT CGATTGCCCA TGAATTGGGC CACAGTCTGG
1151  GTTTGGACCA TGATTCTCCC GGGCACAGCT GTCCCTGTCC AGGTCCAGCC
1201  CCGGCTAAGA GCTGCATCAT GGAGGCCTCC ACAGACTTCC TACCAGGTTT
1251  GAACTTCAGC AACTGCAGCC GACAGGCCCT GGAAAAGGCC CTCCTGGAAG
1301  GAATGGGCAG CTGCCTCTTC GAACGGCAAC CCAGCCTGGC CCCTATGTCC
1351  TCTTTGTGTG GAAATATGTT TGTGGACCCC GGAGAGCAGT GTGACTGTGG
1401  CTTCCCAGAT GAATGCACTG ATCCCTGCTG TGACCATTTC ACCTGCCAGC
1451  TGAGGCCAGG AGCGCAGTGT GCATCTGATG GACCCTGTTG TCAAAACTGC
1501  AAGTTGCACC CAGCTGGTTG GCTGTGCCGC CCTCCCACAG ACGATTGTGA
1551  TCTGCCTGAG TTCTGCCCAG GAGATAGCTC TCAGTGCCCG TCTGACATCA
```

Fig. 20

```
1601  GACTTGGGGA CGGTGAGCCT TGTGCTAGTG GAGAGGCTGT GTGTATGCAT
1651  GGGCGCTGTG CCTCCTATGC CCGGCAGTGC CAGTCACTTT GGGGACCCGG
1701  GGCCCAGCCT GCTGCGCCAC TTTGCCTCCA AACAGCCAAC ACTCGGGGTA
1751  ATGCCTTTGG GAGCTGTGGG CGCAGCCCTG GTGGTAGCTA CATGCCTTGT
1801  GCCCCTAGAG ATGTCATGTG TGGGCAACTG CAGTGCCAGT GGGGTAGGAG
1851  CCAGCCTCTG TTGGGCTCAG TCCAAGATCG GCTCTCGGAG GTCCTGGAAG
1901  CCAACGGGAC ACAGTTAAAC TGCAGCTGGG TGGACCTGGA CCTGGGCAAT
1951  GATGTGGCCC AGCCTCTTCT GGCTCTGCCT GGCACTGCCT GTGGTCCTGG
2001  CCTGGTGTGC ATCGGCCACC GATGCCAGCC CGTGGATCTC CTGGGAGCAC
2051  AGGAATGTCG AAGAAAATGC CACGGCCATG GGGTCTGTGA CAGCAGCGGG
2101  CACTGCCGCT GTGAAGAGGG CTGGGCACCT CCAGACTGCA TGACCCAGCT
2151  CAAAGCAACC AGCTCCCTGA CCACAGGCCT GCTCCTCAGC CTCCTGTTGT
2201  TATTGGTCCT CGTACTACTT GGTGCCAGCT ACTGGCACCG TGCCCGCCTG
2251  CATCAGCGGC TCTGCCAGCT TAAGGGATCC AGCTGCCAAT ATAGGGCACC
2301  CCAATCCTGT CCTCCTGAAC GACCAGGACC TCCACAGCGG GCACAGCAGA
2351  TGACAGGCAC TAAGTCTCAG GGGCCTACCA AACCCCCACC CCCAAGAAAG
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2601  ACTAGAGAAG CAGGAGAACA GACAATCTGG TGTCCAGCCC TAAAGAACCA
2651  CCAGGCGCTG TTAAGCAATA CCTGGGGACG CACTAAAATA GCTGCAGCGG
2701  GATGCTGGGG AGGGGCCGAA GCCGGGGCTG GAGTAGGTGG CGGAAGGACA
2751  TGGACACTGT CTATCTCTGC TCTGTTGTAA TAAATGTGAG ATCTTGGAAA
2801  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA
```

Fig. 20 (con't)

```
  1  TGGCAGTGCT AAGCAGCACT CTACCAGTGA ATTTACCCCC ACACTCCCTG CCTTTTTCNT
 61  TTGTGTGGTT GAATCCTGGG GATGGNAACC CAGGGNACAG CAGTCCCCAG ATCAACTCCC
121  ATCTTCTCAG AGGCACTTTA GGGCMRTGGG GCTGGGCAGC ACTTCATGGG TCCTCAGGCA
181  GTTGGGGCTA ACTGCCTCAG GAAGGCATCC CACTTTGGAG GGCTTCCATC TTTTTGAGGC
241  ACTTTGGGAC AGGGAAAGTG GGTACCATTC TCTCAGGCCT TATGACAATT GGGGTAACTA
301  CGCCAAGCAG GACAGAGGCT GCTGGGGCAG GGTGGCCTTC CCCTCCCCCG GTGTACATAT
361  TGTACCTGTG TACTATTTTG TATATACCGG GGTAGGGACA CCCCTGTATC ATAGTGGAGG
421  TTGGAGCTGG CAAATGGGAA GAGCTTCTAA TAATCACTTT GGGCTGGGAA CCTTATTTAT
481  TGGTAGTGTT AGGTCAGAGG GCAGKAKGCG GAGACAAGGT TGTGGCACCT GCTGATGCAG
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Fig. 21

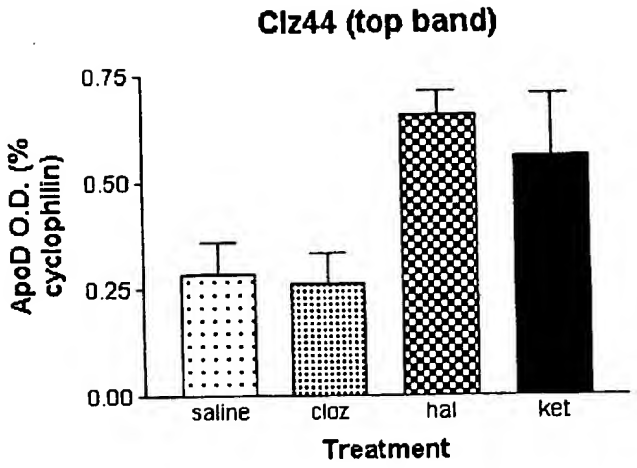

Clz44 (top band)
ApoD O.D. (% cyclophilin)
0.75
0.50
0.25
0.00
saline
cloz
hal
ket
Treatment

Fig. 22

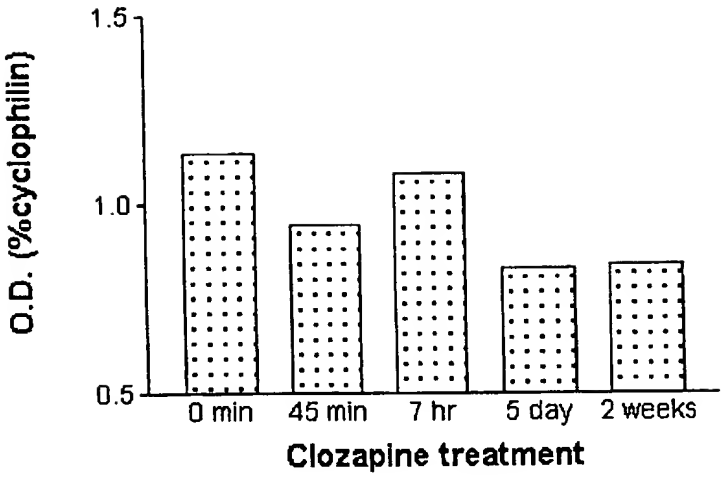

O.D. (%cyclophilin)
1.5
1.0
0.5
0 min
45 min
7 hr
5 day
2 weeks
Clozapine treatment

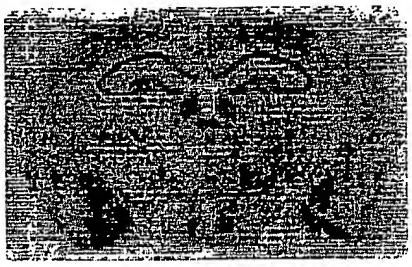
Fig. 23

A

B

Fig. 24

A



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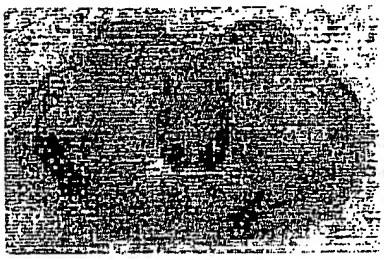


Fig. 25

A

B

Fig. 26

A

B

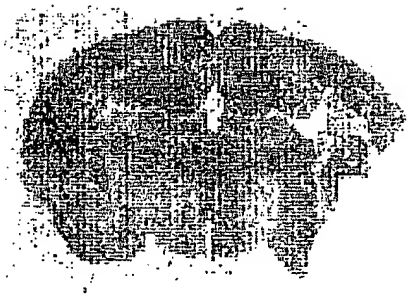
Fig. 27

A

B

Fig. 28

A



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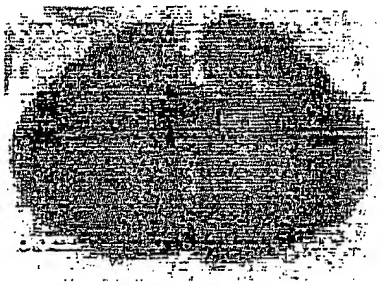


Fig. 29

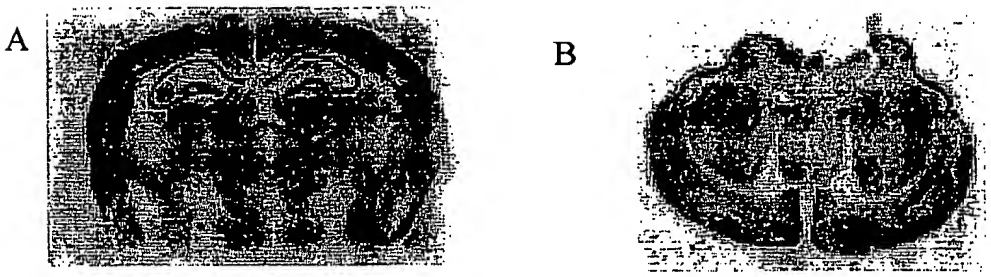


Fig. 30

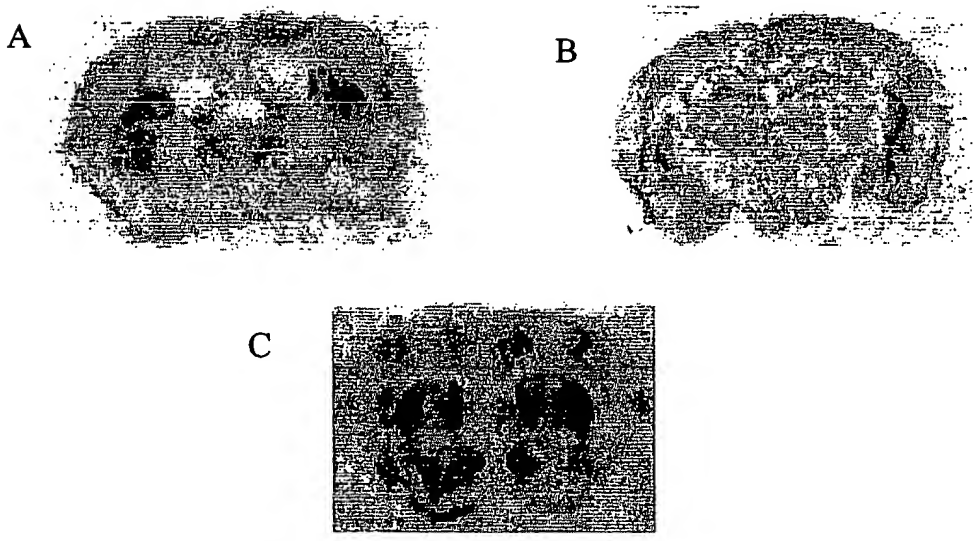


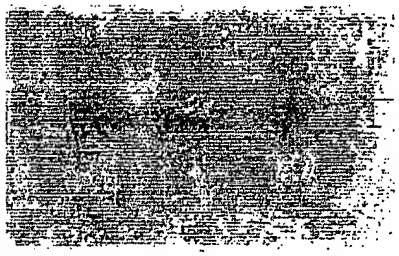
Fig. 31

A

B

Fig. 32

A



B

Fig. 33

SEQUENCE LISTING

<110> Thomas, Elizabeth A
Sutcliffe, J. Gregor
Pribyl, Thomas M
Hilbush, Brian S
Hasel, Karl W

<120> Regulation of Gene Expression by Neuroleptic Agents

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gccgtgactt ggagactatg agaagacaac gaaatgaagt tgtagttgaa ttaaggaaaa 120
a 121

<210> 6
<211> 166
<212> DNA
<213> Mus musculus

<400> 6
cggcggtggc catcagactc tgagacagag agccaatctc acattcaagt gttcaccaac 60
cactgacgtg tttttatttc cttctatatg attttaagat gtgttttctg cattctgtat 120
agaaacatat caaactaaat aaaagcagtg tctttattac caaaaa 166

<210> 7
<211> 343
<212> DNA
<213> Mus musculus

<400> 7
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tttctcccgc attgaaacag tctgtttccc gctgttgact ccatgctata tacatgctat 240
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actggcagaa gctttcacca agcttgcatc agctacacaa aaa 343

<210> 8
<211> 138
<212> DNA
<213> Mus musculus

<400> 8
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agcattctca agtgaggcgt cagcgagctt gcttttctct agtcgttgag aacgaataaa 120
gcttcattgt gagaaaaa 138

<210> 9
<211> 39
<212> DNA
<213> Mus musculus

<400> 9
cggtattcag tggtgatgcc taaaggaatg tcagaaaaa 39

<210> 10
<211> 45

<212> DNA
<213> Mus musculus

<400> 10
cggtccctgc cgctcaataa acatgaactg aacaaacaac aaaaa 45

<210> 11
<211> 56
<212> DNA
<213> Mus musculus

<400> 11
cggtgcattt gttcaggtaa aatctgtgca ataaaataac aaactgtctc caaaaa 56

210> 12
<211> 212
<212> DNA
<213> Mus musculus

<400> 12
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<210> 13
<211> 1156
<212> DNA
<213> Mus musculus

<400> 13
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<210> 14
<211> 118
<212> DNA

<213> Mus musculus

<400> 14
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gtgaattcca gtctaggaag gtctacatag agaaatcctg tctcaaacaa aacaaaaa 118

<210> 15
<211> 122
<212> DNA
<213> Mus musculus

<400> 15
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aa 122

<210> 16
<211> 240
<212> DNA
<213> Mus musculus

<400> 16

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<210> 17
<211> 220
<212> DNA
<213> Mus musculus

<400> 17
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tccaccagag aagtattgcc acaagcaagc cgtccaagta caatcacaga cagcgactct 180
acacaaggaa cagagaatga agtcagaggg cacacaaaaa 220

<210> 18
<211> 319
<212> DNA
<213> Mus musculus

<400> 18
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ccatcccagg tgctggaggc actcagagac tcacccgagc agtcggcaaa tcgctagcaa 180
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<210> 19
<211> 279
<212> DNA
<213> Mus musculus

<400> 19

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gagtgagttc tcgagaacat cactgactcc ggcggtagct tctcttctgt gtgactagca 240
gtgacttcat cttaataaac tgatctgcaa acccaaaaa 279

<210> 20
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: cDNA anchor primer

<400> 20
gaattcaact ggaagcggcc gcaggaattt ttttttttt ttttvnn 48

<210> 21
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' RT primer

<400> 21
aggtcgacgg tatcgg 16

<210> 22
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer

<400> 22
ggtcgacggt atcggn 16

<210> 23
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: universal 3' PCR primer

<400> 23
gagctccacc gcggt 15

<210> 24
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer

<400> 24
cgacggtatc ggnnnn 16

<210> 25
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer with parsing bases C-A-C-C

<400> 25
cgacggtatc ggcacc 16

<210> 26
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-T-G-T

<400> 26
cgacggtatc ggttgt 16

<210> 27
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer with parsing bases T-A-T-T

<400> 27
cgacggtatc ggtatt 16

<210> 28
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_3 clone

<400> 28
gatcgaatcc ggagtacagt gactttgagt 30

<210> 29
<211> 30
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_5 clone

<400> 29
gatcgaatcc ggcaccctac tggatcctgg 30

<210> 30
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_8 clone

<400> 30
gatcgaatcc ggcatccagc tggatgtcag 30

<210> 31
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_10 clone

<400> 31
gatcgaatcc ggccagagtc tgattagggc 30

<210> 32
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_12 clone

<400> 32
gatcgaatcc ggccgtggcg gacaacgaga 30

<210> 33
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_15 clone

<400> 33
gatcgaatcc ggcggtggcc atcagactct 30

<210> 34
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_24 clone

<400> 34
gatcgaatcc ggggcaagga gcaccagaag 30

<210> 35
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_33 clone

<400> 35
gatcgaatcc ggtactccgc tctgatcatc 30

<210> 36
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_34 clone

<400> 36
gatcgaatcc ggtattcagt ggtgatgcct 30

<210> 37
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_37 clone

<400> 37
gatcgaatcc ggtccctgcc gctcaataaa 30

<210> 38
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_38 clone

<400> 38
gatcgaatcc ggtgcatttg ttcaggtaaa 30

<210> 39
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_40 clone

<400> 39
gatcgaatcc ggttgtggtt cagtggcaag 30

<210> 40
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_6 clone

<400> 40
gatcgaatcc ggcacttggg aggcagagac 30

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_16 clone

<400> 41
gatcgaatcc ggctagcagc agaaacgtct 30

<210> 42
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_22 clone

<400> 42
gatcgaatcc gggctagaac gccagccaga 30

<210> 43
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_32 clone

<400> 43
gatcgaatcc ggtacgatgc tgtgacaatt 30

<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_36 clone

<400> 44
gatcgaatcc ggtcatcgca gctgtcaatg 30

<210> 45
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_42 clone

<400> 45
gatcgaatcc ggtgacagac agaagaggat 30

<210> 46
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_17 clone

<400> 46
gatcgaatcc ggctcagcac ttggcagctg 30

<210> 47
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_26 clone

<400> 47
gatcgaatcc ggggctggag taggtggcgg 30

<210> 48

<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_28 clone

<400> 48
gatcgaatcc ggggtaggga cacccctgta 30

<210> 49
<211> 155
<212> DNA
<213> Mus musculus

<400> 49
cggctcagca cttggcagct gtcccgtgcg gggactcagt ccaactctgt gtttgctttt 60
cttcttggcc aaagcatgtg ccactaagct gtcctggagg acattgtctt tatgaaacac 120
acctggaata aaaccacttc ttacatgtcc aaaaa 155

<210> 50
<211> 80
<212> DNA
<213> Mus musculus

<400> 50
cggggctgga gtaggtggcg gaaggacatg gacactgtct atctctgctc tgttgtaata 60
aatgtgagat cttggaaaaa 80

<210> 51
<211> 206
<212> DNA
<213> Mus musculus

<400> 51
cggggtaggg acacccctgt atcatagtgg aggttggagc tggcaaatgg gaagagcttc 60
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gcggagacaa ggttgtggca cctgctgatg cagctcctct ttattttgct ttttacttgg 180
gaaataaatg gatttagcca taaaaa 206

<210> 52
<211> 206
<212> DNA
<213> Mus musculus

<400> 52

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<210> 53
<211> 537
<212> DNA

<213> Mus musculus

<400> 53

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<210> 54
<211> 2833
<212> DNA
<213> Mus musculus

<400> 54

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<210> 55
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<212> DNA
<213> Mus musculus

<400> 55

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<210> 56
<211> 1603
<212> DNA
<213> Mus musculus

<400> 56

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tgcgcgagga attgagccag cgactggggc tctctgaggc ccgagtacag gtttggtttc 780
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gagccgctag ccagtttgaa gcttgtagag ttgcacccta tgtcaacgta ggtgctttaa 900
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cgcagctgca gctggacagc gccgtggcgc acgcgcacca ccacctgcat ccgcacctgg 1020
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<210> 57
<211> 271
<212> DNA
<213> Mus musculus

<400> 57
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catgtttgct gtgtatctgc ttcagagact tcagggtgtt tgcccawwrr gttgtctgac 180
cttttattat gaaggtttac aagtttgtta tgcattctag ataaaagttc ctttgtgtca 240
gatgaatcac ataaaaattt tcctccaaaa a 271

<210> 58
<211> 411
<212> DNA
<213> Mus musculus

<400> 58
cggctaatat tgataatctt tatttgaaaa aatgtcatga accatttgaa tgatgagcca 60
cagaacctca gttgaattta tttccacttt tggcatgtta aatatagatt taattttaag 120
tacttcaatt aatgggttta taaagtcaag cactagcatt ggtcagtttt gtatgatagg 180
atgtaagtgt gttctcacct gcagtgtaaa tacagcacac tgtagaattc tcttaaggtg 240
catagtaaat gtatagatag tcacaggcgg ttttgtaatg tatacatttc taatctatta 300
ttcctaacct gtcatgtttg cagagagaaa agaatttttc taatgatctg taaaattatg 360
ttaacttcta caagtaggta ttctaaataa acttttttaa aagaccaaaa a 411

<210> 59
<211> 295
<212> DNA
<213> Mus musculus

<400> 59
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agcaggagta gaaccccatt tcgcctgtgt gagaaagatc gaatggagct actagaaata 120
gcaaaagcca acgcagcaaa agctctggga acagccaact tcgacttgcc agcaagtctc 180
cgagccaagg aggcaagcca ggggacagct gtttccagca gtgggccaaa ggtggagcat 240
tcagaaaagc agactgaaga tacaactaaa aataccagtg aaaagtcttc tacac 295

<210> 60
<211> 84
<212> DNA
<213> Mus musculus

<400> 60

cggaatactg aggaggaagg acccaagtac aagtccaaag tttcattaaa aggcaataga 60
gaaagtgatg gatttagaga aaaa 84

<210> 61
<211> 42
<212> DNA
<213> Mus musculus

<400> 61

cggccggcat gaaataaaac atttaaatag tgctggcaaa aa 42

<210> 62
<211> 397
<212> DNA
<213> Mus musculus

<400> 62

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gtgacggatg agtataagaa tgatgtaaaa aacagatctg tttatattaa aggtttccca 180
actgacgcca cccttgatga tataaaagaa tggctagacg ataaaggcca aatactgaat 240
attcaaatga gaagaacatt acacaaaaca tttaaggggt caatatttgc tgtgtttgat 300
agtattcagt ctgcaaagaa gtttgtggag atccctggcc agaagtacaa agacactaac 360
ctgctaatac tctttaagga agattacttt gcaaaaa 397

<210> 63
<211> 240
<212> DNA
<213> Mus musculus

<400> 63

cggccgtggt ggcgcacacc attaatccca gcactcagga ggcagaggca ggcggatttc 60
tgagttcgag gccagcctgg tctacagagt gagttccagg acagccaggg ctacacagag 120
aaaccctgtc ttgaagaaac aaaaaggtta ggctagtatt tggagaaaga agattagaaa 180
atggaagtga aagacgaaga agacatacag gaaggtgaag aaaaagctgt tagagaaaaa 240

<210> 64
<211> 196
<212> DNA
<213> Mus musculus

<400> 64

cggcatgggt ggtcttcatc ctggccgata gctgcagaac tgatgtgaat gtaccttcat 60
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agcaaagctg cctcccccgt cttgatttcc tgttgatttc tattctataa ttgaacaggc 180
atttctgtgg caaaaa 196

<210> 65
<211> 95
<212> DNA
<213> Mus musculus

<400> 65

cggcagacct agctcagctt gatggggtgt gacaactgca attagaggca agccgcctgc 60

tgcccccaga gcattaagag caaattggag aaaaa 95

<210> 66
<211> 343
<212> DNA
<213> Mus musculus

<400> 66

cggggcaagg agcaccagaa gaacagcagc caacggccag gagcaggcac catggttctg 60
ctgcagcggg agctggctca ggaagacagc ctcaacaagc tggctctcca gtatggctgc 120
aaacactcag agtgattaac agtggcaaag taagttggaa agcgtttctg gaatcgctct 180
tttctcccgc attgaaacag tctgtttccc gctgttgact ccatgctata tacatgctat 240
atacatgctg tatacatgct atatacatgc tatatagtac atgctataat catgctatat 300
actggcagaa gctttcacca agcttgcatc agctacacaa aaa 343

<210> 67
<211> 273
<212> DNA
<213> Mus musculus

<400> 67

cgggccccat caatttcacc atgttcctca ccatgtttgg ggagaagcta aacggcactg 60
accccgagga cgtcatcaga aacgccttcg cttgctttga tgaggaagcc acaggcacca 120
tccaggagga ttacctgagg gagcccctga ccaccatggg cgaccgcttc acagacgagg 180
aagtggatga gctgtacaga gaggccccca ttgacaaaaa ggggaacttc aactacattg 240
agttcacacg catcctgaag cacggcgcaa aaa 273

<210> 68
<211> 273
<212> DNA
<213> Mus musculus

<400> 68

cgggcgccat caatttcacc atgttcctca ccatgtttgg ggagaagcta aacggcactg 60
accccgagga cgtcatcaga aacgccttcg cttgctttga tgaggaagcc acaggcacca 120
tccaggagga ttacctgagg gagctgctga ccaccatggg cgaccgcttc acagacgagg 180
aagtggatga gctgtacaga gaggccccca ttgacgaaaa ggggaacttc aactacattg 240
agttcacacg catcctgaag cacggcgcaa aaa 273

<210> 69
<211> 115
<212> DNA
<213> Mus musculus

<400> 69

cggggctcaa agacaagggt tcgagtcccg ctcctgccca cgcccactgc attcgggctt 60
cagtttttcc ttctctgaaa tggggacgtg gataaaatca tcttcaaagc aaaaa 115

<210> 70
<211> 335
<212> DNA

<213> Mus musculus

<400> 70

cggtcatcgc agctgtcaat ggttatgctc ttggtggggg ttgtgaactt gccatgatgt 60
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ccatcccagg tgctggaggc actcagagac tcacccgagc agtcggcaaa tcgctagcaa 180
tggagatggt cctcactggt gaccacatct cagctcagga tgcaaagcag gcaggtcttg 240
taagcaagat ttttcctgtt gaaaaactgg ttgaagaagc catccaatgt gcagaaaaaa 300
ttgccagcaa ttctaaagtc gtagtagcca tggcg 335

<210> 71
<211> 240
<212> DNA
<213> Mus musculus

<400> 71
cggtatgtgg gtagagtggt ccattcgttt gatggcacga aggaagcagc agctgctttg 60
gttgacttgg gcctttatat aggatttaat ggttgctctc tgaaaactga agctaacttg 120
gaagttctga agtcaatacc tagtgaaaaa ctaatgattg agacagatgc accttggtgt 180
ggagttaaaa gtacacatgc tggatcaaaa tacataaacc cttgggtttc cctccaaaaa 240

<210> 72
<211> 107
<212> DNA
<213> Mus musculus

<400> 72

cggtatccac agtaaaattg tgagtagctt aatctgttta tctccattac aattcctctg 60
caactatttt ccttgatgtt gtaataaaaa ggaggtagga tgaaaaa 107

<210> 73
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA
primer for CLZ_18 clone

<400> 73

gatcgaatcc ggctgcaggt gagggctggt 30

<210> 74
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA
primer for CLZ_43 clone

<400> 74

gatcgaatcc ggctaatatt gataatcttt 30

<210> 75
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_44 clone

<400> 75

gatcgaatcc ggacggtgta ccccgaggat 30

<210> 76
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_47 clone

<400> 76

gatcgaatcc ggaatactga ggaggaagga 30

<210> 77
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_48 clone

<400> 77

gatcgaatccg gccggcatg aaataaaaca 30

<210> 78
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_49 clone

<400> 78

gatcgaatcc ggctgacaac agactttaat 30

<210> 79
<211> 30
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_50 clone

<400> 79

gatcgaatcc ggccgtggtg gcgcacacca 30

<210> 80
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_51 clone

<400> 80

gatcgaatcc ggcatgggtg gtcttcatcc 30

<210> 81
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_52 clone

<400> 81

gatcgaatcc ggcagaccta gctcagcttg 30

<210> 82
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_56 clone

<400> 82

gatcgaatcc gggccccatc aatttcacca 30

<210> 83
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_57 clone

<400> 83

gatcgaatcc gggcgccatc aatttcacca 30

<210> 84
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_60 clone

<400> 84

gatcgaatcc ggggctcaaa gacaagggtt 85

<210> 85
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_62 clone

<400> 84

gatcgaatcc ggtatgtggg tagagtggtc 30

<210> 86
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_64 clone

<400> 86

gatcgaatcc ggtcatcgca gctgtcaatg 30

<210> 87
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended-TOGA primer for CLZ_65 clone

<400> 87

gatcgaatcc ggtatccaca gtaaaattgt 30

<210> 88
<211> 21
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: probe for screening human brain library

<400> 88

aacaagtccg tcctggcatg g 21

<210> 89
<211> 59
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' adapter primer for direct sequencing

<400> 89

tcccagtcac gacgttgtaa aacgacggct catatgaatt aggtgaccga cggtatcgg 59

<210> 90
<211> 46
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 3' adapter primer for direct sequencing

<400> 90

cagcggataa caatttcaca cagggagctc caccgcggtg gcggcc 46

<210> 91
<211> 23
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 5' sequencing primer for direct sequencing

<400> 91

cccagtcacg acgttgtaaa acg 23

<210> 92
<211> 19
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 3' sequencing primer for direct sequencing

<400> 92

ttttttttttt ttttttttv 19

<210> 93
<211> 35
<212> DNA
<213> Artificial Sequence

<223> Description of Artificial Sequence: 3' sequencing primer for direct sequencing

<400> 93

ggtggcggcc gcaggaattt ttttttttttt ttttt 35

<210> 94
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5' PCR primer with parsing bases A-G-T-A

<400> 94
cgacggtatc ggagta

<210> 95
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Extended TOGA primer for CLZ_58

<400> 95

gatcgaatcc gggatcccac gagggccacc 30

<210> 96
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_44 with parsing bases A-C-G-G

<400> 96

cgacggtatc ggacgg 16

<210> 97
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_38 with parsing bases T-G-C-A

<400> 97

cgacggtatc ggtgca 16

<210> 98
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_16 with parsing bases C-T-A-G

<400> 98

cgacggtatc ggctag 16

<210> 99
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_17 with parsing bases C-T-C-A

<400> 99

cgacggtatc ggctca 16

<210> 100
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_24 with parsing bases G-G-C-A

<400> 100

cgacggtatc ggggca 16

<210> 101
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_26 with parsing bases G-G-C-T

<400> 101

cgacggtatc ggggct 16

<210> 102
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_28 with parsing bases G-G-T-A

<400> 102

cgacggtatc ggggta 16

<210> 103
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_34 with parsing bases T-A-T-T

<400> 103

cgacggtatc ggtatt 16

<210> 104
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_43 with parsing bases C-T-A-A

<400> 104

cgacggtatc ggctaa 16

<210> 105
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_44 with parsing bases A-C-G-G

<400> 105

cgacggtatc ggacgg 16

<210> 106
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 5'PCR primer for CLZ_64 with parsing bases T-C-A-T

<400> 106

cgacggtatc ggtcat 16

<210> 107
<211> 1717
<212> DNA
<213> Homo sapiens

<400> 107

gatattttaa aaattgcata catatgaaat atattgggat ttacaaacaa ctaaacacat 60
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aagaaagtag caaaaggcta ttcctcccca gaaccgg 1717

<210> 108
<211> 1717

<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (562)..(1716)

<400> 108
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gattttgaag taggatattg cttgatgtca ttgccaggtg caaagttgca aggaaacgtg 180

agtttataac tttgttattg ccagtgcatc atagaagtaa atgtagtata aaataatagg 240

ctataatatt tttgtagtgg atctcttgta tatattgttc actttgatgt ctttttcagc 300

taccctttt tacctaagtt ttatagctat agattttatt attatttttt gtttccacat 360

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ttgaaacaag tccgtcctgg c atg gac cta tcc aag gtg gtt ctg cct aca 591
Met Asp Leu Ser Lys Val Val Leu Pro Thr
1 5 10

ttt att ttg gaa ccc cgt tct ttc ctg gat aaa ctt tca gat tac tac 639
Phe Ile Leu Glu Pro Arg Ser Phe Leu Asp Lys Leu Ser Asp Tyr Tyr
15 20 25

tat cat gca gat ttc cta tct gag gca gct ctt gaa gaa aat cct tat 687
Tyr His Ala Asp Phe Leu Ser Glu Ala Ala Leu Glu Glu Asn Pro Tyr
30 35 40

ttc cgt ttg aag aaa gta gtg aaa tgg tat ttg tca gga ttc tat aaa 735
Phe Arg Leu Lys Lys Val Val Lys Trp Tyr Leu Ser Gly Phe Tyr Lys
45 50 55

aag cca aag gga ctg aag aaa cct tat aat cct ata ctt ggc gag act 783
Lys Pro Lys Gly Leu Lys Lys Pro Tyr Asn Pro Ile Leu Gly Glu Thr
60 65 70

ttc cgt tgt tta tgg att cat ccc aga aca aac agc aaa act ttt tat 831
Phe Arg Cys Leu Trp Ile His Pro Arg Thr Asn Ser Lys Thr Phe Tyr
75 80 85 90

att gct gaa cag gtg tcc cat cat cca cca ata tct gcc ttt tat gtt 879
Ile Ala Glu Gln Val Ser His His Pro Pro Ile Ser Ala Phe Tyr Val
95 100 105

agt aat cga aaa gat gga ttt tgc ctt agc ggt agt atc ctg gct aag 927
Ser Asn Arg Lys Asp Gly Phe Cys Leu Ser Gly Ser Ile Leu Ala Lys

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                110                 115                 120

tct aag ttt tat gga aac tca tta tct gca ata tta gag gga gaa gca      975
Ser Lys Phe Tyr Gly Asn Ser Leu Ser Ala Ile Leu Glu Gly Glu Ala
        125                 130                 135

cgg tta act ttc ttg aat aga ggt gaa gat tat gta atg aca atg cca     1023
Arg Leu Thr Phe Leu Asn Arg Gly Glu Asp Tyr Val Met Thr Met Pro
    140                 145                 150

tac gct cat tgt aaa gga att ctt tat ggt aca atg aca ctg gag ctt     1071
Tyr Ala His Cys Lys Gly Ile Leu Tyr Gly Thr Met Thr Leu Glu Leu
155                 160                 165                 170

ggt gga aca gtc aat att aca tgt caa aaa act gga tac agt gca ata     1119
Gly Gly Thr Val Asn Ile Thr Cys Gln Lys Thr Gly Tyr Ser Ala Ile
                175                 180                 185

ctt gaa ttt aaa cta aag cca ttc cta ggg agt agt gac tgt gtt aat     1167
Leu Glu Phe Lys Leu Lys Pro Phe Leu Gly Ser Ser Asp Cys Val Asn
            190                 195                 200

caa ata tca ggg aaa ctt aaa ctg gga aaa gaa gtc cta gct act ttg     1215
Gln Ile Ser Gly Lys Leu Lys Leu Gly Lys Glu Val Leu Ala Thr Leu
        205                 210                 215

gaa ggt cat tgg gat agt gaa gtt ttt att act gat aaa aag act gat     1263
Glu Gly His Trp Asp Ser Glu Val Phe Ile Thr Asp Lys Lys Thr Asp
    220                 225                 230

aat tca gag gtt ttc tgg aat cca aca cct gay att aag caa tgg aga     1311
Asn Ser Glu Val Phe Trp Asn Pro Thr Pro Asp Ile Lys Gln Trp Arg
235                 240                 245                 250

tta ata agg cac act gta aaa ttt gaa gaa cag gga gat ttt gaa tca     1359
Leu Ile Arg His Thr Val Lys Phe Glu Glu Gln Gly Asp Phe Glu Ser
                255                 260                 265

gag aaa ctc tgg caa cgg gta act cga gcc ata aat gcc aaa gac caa     1407
Glu Lys Leu Trp Gln Arg Val Thr Arg Ala Ile Asn Ala Lys Asp Gln
            270                 275                 280

act gaa gct acc caa gag aag tat gtt ttg gaa gaa gct caa aga caa     1455
Thr Glu Ala Thr Gln Glu Lys Tyr Val Leu Glu Glu Ala Gln Arg Gln
        285                 290                 295

gct gcc agg gat cgg aaa aca aaa aat gaa gag tgg tct tgc aaa tta     1503
Ala Ala Arg Asp Arg Lys Thr Lys Asn Glu Glu Trp Ser Cys Lys Leu
    300                 305                 310

ttt gaa ctt gat cca ctc aca gga gaa tgg cat tac aag ttt gca gat     1551
Phe Glu Leu Asp Pro Leu Thr Gly Glu Trp His Tyr Lys Phe Ala Asp
315                 320                 325                 330

acc cga cca tgg gac cca ctt aat gat atg ata cag ttt gaa aaa gat     1599
Thr Arg Pro Trp Asp Pro Leu Asn Asp Met Ile Gln Phe Glu Lys Asp
                335                 340                 345
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ggt gtt att cag acc aaa gtg aaa cat cgt act cca atg gtt agc gtc    1647
Gly Val Ile Gln Thr Lys Val Lys His Arg Thr Pro Met Val Ser Val
            350                 355                 360

ccc aaa atg aaa cat aag cca acc agg caa cag aag aaa gta gca aaa    1695
Pro Lys Met Lys His Lys Pro Thr Arg Gln Gln Lys Lys Val Ala Lys
        365                 370                 375

ggc tat tcc tcc cca gaa ccg g                                     1717
Gly Tyr Ser Ser Pro Glu Pro
    380                 385
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<210> 109

<211> 385

<212> PRT

<213> Homo sapiens

<400> 109

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Met Asp Leu Ser Lys Val Val Leu Pro Thr Phe Ile Leu Glu Pro Arg
1               5                   10                  15

Ser Phe Leu Asp Lys Leu Ser Asp Tyr Tyr Tyr His Ala Asp Phe Leu
            20                  25                  30

Ser Glu Ala Ala Leu Glu Glu Asn Pro Tyr Phe Arg Leu Lys Lys Val
        35                  40                  45

Val Lys Trp Tyr Leu Ser Gly Phe Tyr Lys Lys Pro Lys Gly Leu Lys
    50                  55                  60

Lys Pro Tyr Asn Pro Ile Leu Gly Glu Thr Phe Arg Cys Leu Trp Ile
65                  70                  75                  80

His Pro Arg Thr Asn Ser Lys Thr Phe Tyr Ile Ala Glu Gln Val Ser
                85                  90                  95

His His Pro Pro Ile Ser Ala Phe Tyr Val Ser Asn Arg Lys Asp Gly
            100                 105                 110

Phe Cys Leu Ser Gly Ser Ile Leu Ala Lys Ser Lys Phe Tyr Gly Asn
        115                 120                 125
```

```
Ser Leu Ser Ala Ile Leu Glu Gly Glu Ala Arg Leu Thr Phe Leu Asn
    130                 135                 140

Arg Gly Glu Asp Tyr Val Met Thr Met Pro Tyr Ala His Cys Lys Gly
145                 150                 155                 160

Ile Leu Tyr Gly Thr Met Thr Leu Glu Leu Gly Gly Thr Val Asn Ile
                165                 170                 175

Thr Cys Gln Lys Thr Gly Tyr Ser Ala Ile Leu Glu Phe Lys Leu Lys
            180                 185                 190

Pro Phe Leu Gly Ser Ser Asp Cys Val Asn Gln Ile Ser Gly Lys Leu
        195                 200                 205

Lys Leu Gly Lys Glu Val Leu Ala Thr Leu Glu Gly His Trp Asp Ser
    210                 215                 220

Glu Val Phe Ile Thr Asp Lys Lys Thr Asp Asn Ser Glu Val Phe Trp
225                 230                 235                 240

Asn Pro Thr Pro Asp Ile Lys Gln Trp Arg Leu Ile Arg His Thr Val
                245                 250                 255

Lys Phe Glu Glu Gln Gly Asp Phe Glu Ser Glu Lys Leu Trp Gln Arg
            260                 265                 270

Val Thr Arg Ala Ile Asn Ala Lys Asp Gln Thr Glu Ala Thr Gln Glu
        275                 280                 285

Lys Tyr Val Leu Glu Glu Ala Gln Arg Gln Ala Ala Arg Asp Arg Lys
    290                 295                 300

Thr Lys Asn Glu Glu Trp Ser Cys Lys Leu Phe Glu Leu Asp Pro Leu
305                 310                 315                 320

Thr Gly Glu Trp His Tyr Lys Phe Ala Asp Thr Arg Pro Trp Asp Pro
                325                 330                 335

Leu Asn Asp Met Ile Gln Phe Glu Lys Asp Gly Val Ile Gln Thr Lys
            340                 345                 350
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Val Lys His Arg Thr Pro Met Val Ser Val Pro Lys Met Lys His Lys
355 360 365

Pro Thr Arg Gln Gln Lys Lys Val Ala Lys Gly Tyr Ser Ser Pro Glu
370 375 380

Pro
385

<210> 110

<211> 472

<212> PRT

<213> Homo sapiens

<400> 110

Met Asp Leu Ser Lys Val Val Leu Pro Thr Phe Ile Leu Glu Pro Arg
1 5 10 15

Ser Phe Leu Asp Lys Leu Ser Asp Tyr Tyr Tyr His Ala Asp Phe Leu
20 25 30

Ser Glu Ala Ala Leu Glu Glu Asn Pro Tyr Phe Arg Leu Lys Lys Val
35 40 45

Val Lys Trp Tyr Leu Ser Gly Phe Tyr Lys Lys Pro Lys Gly Leu Lys
50 55 60

Lys Pro Tyr Asn Pro Ile Leu Gly Glu Thr Phe Arg Cys Leu Trp Ile
65 70 75 80

His Pro Arg Thr Asn Ser Lys Thr Phe Tyr Ile Ala Glu Gln Val Ser
85 90 95

His His Pro Pro Ile Ser Ala Phe Tyr Val Ser Asn Arg Lys Asp Gly
100 105 110

Phe Cys Leu Ser Gly Ser Ile Leu Ala Lys Ser Lys Phe Tyr Gly Asn
115 120 125

Ser Leu Ser Ala Ile Leu Glu Gly Glu Ala Arg Leu Thr Phe Leu Asn
130 135 140

Arg Gly Glu Asp Tyr Val Met Thr Met Pro Tyr Ala His Cys Lys Gly
145 150 155 160

Ile Leu Tyr Gly Thr Met Thr Leu Glu Leu Gly Gly Thr Val Asn Ile
165 170 175

```
Thr Cys Gln Lys Thr Gly Tyr Ser Ala Ile Leu Glu Phe Lys Leu Lys
            180                 185                 190

Pro Phe Leu Gly Ser Ser Asp Cys Val Asn Gln Ile Ser Gly Lys Leu
        195                 200                 205

Lys Leu Gly Lys Glu Val Leu Ala Thr Leu Glu Gly His Trp Asp Ser
    210                 215                 220

Glu Val Phe Ile Thr Asp Lys Lys Thr Asp Asn Ser Glu Val Phe Trp
225                 230                 235                 240

Asn Pro Thr Pro Asp Ile Lys Gln Trp Arg Leu Ile Arg His Thr Val
                245                 250                 255

Lys Phe Glu Glu Gln Gly Asp Phe Glu Ser Glu Lys Leu Trp Gln Arg
            260                 265                 270

Val Thr Arg Ala Ile Asn Ala Lys Asp Gln Thr Glu Ala Thr Gln Glu
        275                 280                 285

Lys Tyr Val Leu Glu Glu Ala Gln Arg Gln Ala Ala Arg Asp Arg Lys
    290                 295                 300

Thr Lys Asn Glu Glu Trp Ser Cys Lys Leu Phe Glu Leu Asp Pro Leu
305                 310                 315                 320

Thr Gly Glu Trp His Tyr Lys Phe Ala Asp Thr Arg Pro Trp Asp Pro
                325                 330                 335

Leu Asn Asp Met Ile Gln Phe Glu Lys Asp Gly Val Ile Gln Thr Lys
            340                 345                 350

Val Lys His Arg Thr Pro Met Val Ser Val Pro Lys Met Lys His Lys
        355                 360                 365

Pro Thr Arg Gln Gln Lys Lys Val Ala Lys Gly Tyr Ser Ser Pro Glu
    370                 375                 380

Pro Asp Ile Gln Asp Ser Ser Gly Ser Glu Ala Gln Ser Val Lys Pro
385                 390                 395                 400

Ser Thr Arg Arg Lys Lys Gly Ile Glu Leu Gly Asp Ile Gln Ser Ser
                405                 410                 415

Ile Glu Ser Ile Lys Gln Thr Gln Glu Glu Ile Lys Arg Asn Ile Met
            420                 425                 430

Ala Leu Arg Asn His Leu Val Ser Ser Thr Pro Ala Thr Asp Tyr Phe
        435                 440                 445

Leu Gln Gln Lys Asp Tyr Phe Ile Ile Phe Leu Leu Ile Leu Leu Gln
    450                 455                 460

Val Ile Ile Asn Phe Met Phe Lys
465                 470
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